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Growth, Thyroid Function, and Sexual Maturation in Down Syndrome

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During the past three decades advances in the biomedical sciences have brought about more effective approaches in the care of children with Down syndrome. This article focuses on new information relating to longitudinal growth, thyroid disorders, and sexual development in these individuals.

Longitudinal Growth

Previous studies suggest that stature and growth rate are reduced at most ages from birth to adolescence in persons with Down syndrome.[1,2] We have evaluated heights and weights from birth to 18 years in children with this syndrome.[3,4] Compared with growth data from the National Center for Health Statistics, children of either sex with Down syndrome were significantly smaller at all age intervals. The mean stature for girls with Down syndrome was reduced from the normal mean by 1.5 to 2.5 SD until 12 years of age, and by more than 3 SD from 12 to 17 years, according to different series. Mean stature for boys was reduced by 2 to 3 SD until 13 years and by 2 to 4 SD thereafter.[4]

Centile charts for 1-month- to 18-year-old children with Down syndrome were constructed from these data. Figures 1 and 2 portray these data for boys and girls, respectively, from 2 to 18 years. For all centiles of children with Down syndrome, stature was less than the equivalent centiles for the National Center for Health Statistics data over the entire age range. The growth charts give smoothed values for five centiles for stature for each sex and two age intervals, from 1 to 36 months and from 2 to 18 years. The centiles for stature reflect the expected smaller size and slower growth rate of children with Down syndrome. It was observed that deficiencies in growth velocity occur at varying times in children with Down syndrome and are of widely different magnitude, particularly in infancy. Thus, compared with normal children, we emphasize that a child with Down syndrome may at various times appear in very different centile levels on these charts.

These studies also revealed that boys were significantly longer and heavier than girls from 3 to 24 months and taller and heavier than girls again after 13 years of age. Differences in the intervening period were not significant. Differences in mean height for those children without congenital heart disease from those with moderate or severe congenital heart disease were approximately +2 cm in boys and +1.5 cm in girls until about 8 years. Growth rate was reduced approximately 20% during infancy in each sex, but only about 5% between 3 and 10 years in girls, and 10% between 3 and 12 years in boys. During the remainder of the growing period, reduction in growth rate was 27% for girls and 50% for boys. This indirectly supports the observation that the adolescent growth spurt in youngsters with Down syndrome is less marked than in normal children. Rarick and Seefeldt[5] observed that average growth velocity during adolescence was somewhat reduced in a population with Down syndrome, but peak height velocity was achieved at an age similar to that of the control group. These investigators also measured sitting height and found that the reduction in stature was largely due to a reduction in lower segment length throughout the period of the study (8 to 18 years).

Although we were unable to determine growth velocities in our cohort, "pseudovelocities" (defined as the mean value for an age interval subtracted from the mean of the subsequent age interval) were computed and compared with reference data from the Fels Research Institute. We observed that the growth pseudovelocities fell between the 10th and 25th centiles of normal girls from 2 to about 13 years, and between the 3rd and

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25th centiles for boys from 2 to 11 years. For the periods ending at 14 to 15 years for girls and at 12 to 13 years for boys, pseudovelocities for stature were between the 50th and 90th centiles.

Anneren and co-investigators[6] studied the growth and somatomedin responses to growth hormone (GH) in three girls and two boys with Down syndrome whose height was less than 3 SD below mean for age. All five children had normal GH responses to arginine-insulin and were treated with human growth hormone (hGH) for 6 months (0.5 U/kg/wk in three divided doses). During this time, growth velocity increased in all subjects, from a range of 2.3 to 2.8 cm per 6 months to 3.3 to 5.8 cm, or 50% to 200%. Serum concentrations of insulin-like growth factor I were low before therapy and increased during treatment. The authors concluded that children with Down syndrome respond to this dose of hGH with an increase in growth velocity, although this increase is not as much as that observed in otherwise normal children with GH deficiency.

Thyroid Dysfunction

The most often observed thyroid abnormality in Down syndrome is hypothyroidism.[7-9] However, hyperthyroidism and thyroiditis without hypothyroidism have been reported.[10-13] Moreover, associations of hypothyroidism and diabetes mellitus,[14] hypothyroidism and precocious sexual development,[15] and autoimmune hypothyroidism and hypoparathyroidism[16] have also been observed in Down syndrome. Autoimmune disease in Down syndrome primarily affects the thyroid.

In a recent study we compared serum thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3), free triiodothyronine (FT3), triiodothyronine uptake (T3U), thyroid-stimulating hormone (TSH), and thyroxine-binding globulin (TBG) levels in 181 individuals with Down syndrome with those in 163 controls. We found a significant difference between the two groups for T4, T3, and TSH levels: T4 was significantly lower and T3 and TSH levels were significantly higher in the Down syndrome population (Table 1). Of the 181 patients with Down syndrome, 29 (16%) showed evidence of hypothyroidism. Of these 29, 25 had elevated serum TSH and 11 had reduced serum T4 concentrations. We also observed a significantly low T4 in four patients in whom the TSH concentration was not raised. Only one patient had a significantly elevated T4 level.[17] When the individuals with Down syndrome were divided into 5-year age groups, there were statistically significant differences between most of the age groups in the different thyroid function categories. In particular, a gradual decline of the mean T4, FT4, T3, FT3, and TBG values was observed with advancing age. As expected, thyroid microsomal autoantibody titers also were significantly inversely correlated with T4, FT4, T3, and FT3. Thyroglobulin autoantibody titers showed a significant inverse correlation with T4 and FT4 and a positive correlation with TSH.

In a previous study[18] we investigated the relationship between thyroid function and mental ability in persons with Down syndrome. Intellectual function in patients with both abnormally high TSH and very low T4 levels was significantly lower (mean IQ, 42) than in patients with Down syndrome with increased TSH values only (mean IQ, 54), or in patients with Down syndrome who had normal thyroid

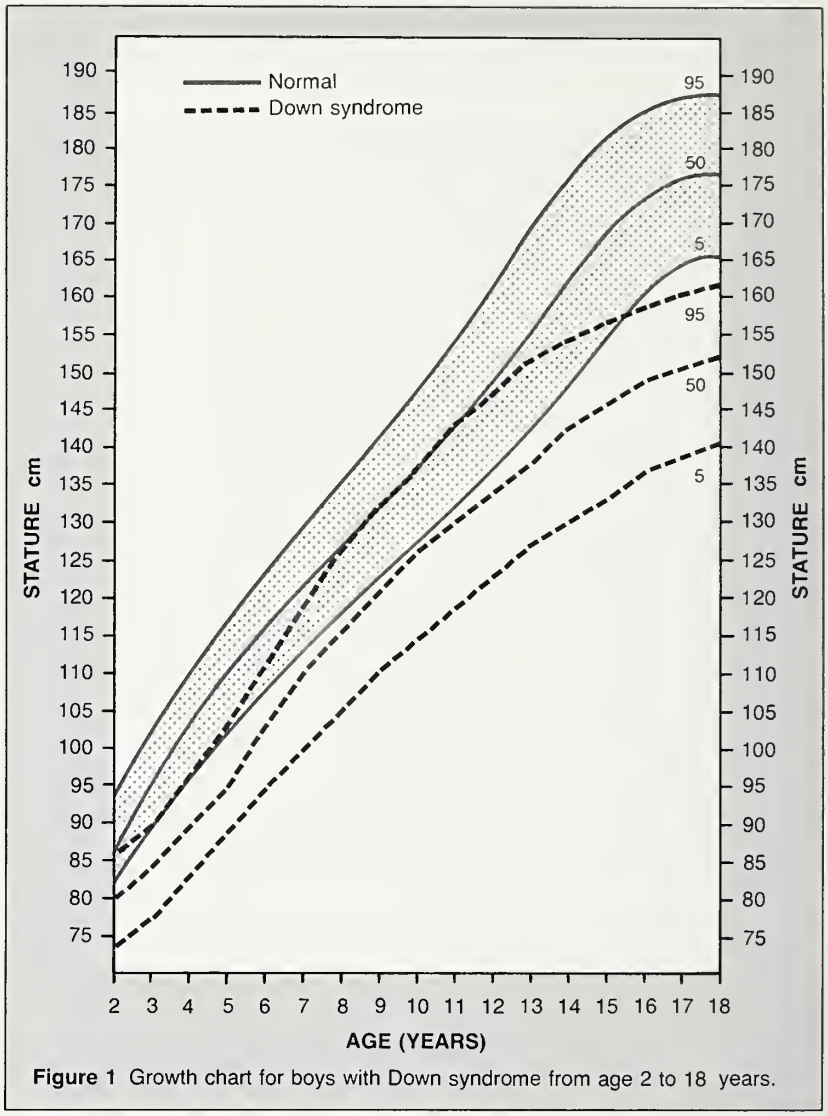

Figure 1 Growth chart for boys with Down syndrome from age 2 to 18 years.

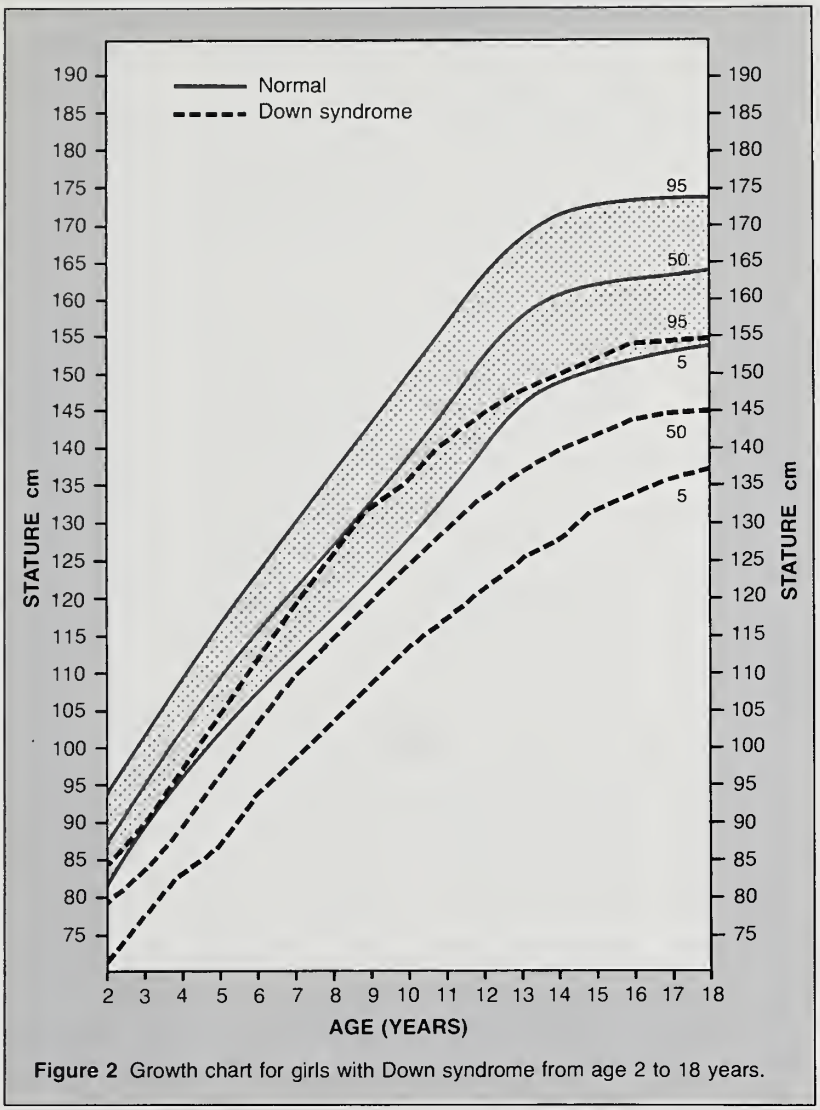

Figure 2 Growth chart for girls with Down syndrome from age 2 to 18 years.

function (mean IQ, 55). It is possible that a decline in IQ in persons with Down syndrome is related to hypothyroidism.[19]

Many features of hypothyroidism are similar to those seen in Down syndrome, which makes it difficult at times to diagnose hypothyroidism clinically in these patients. Because hypothyroidism may compromise normal central nervous system functioning and because clinical symptoms of hypothyroidism are sometimes interpreted as being part of the "Down syndrome gestalt," thyroid function studies, including thyroid antibodies, should be obtained in persons with Down syndrome at regular intervals. Particularly during adolescence and adulthood, annual screening for thyroid dysfunction is recommended. Early detection of thyroid hormone dysfunction and prompt hormone treatment, if hypothyroidism is present, may prevent further cognitive decline and enhance growth and development.

Sexual Maturation

Because the few reports available on sexual maturation in Down syndrome individuals deal primarily with those who have been institutionalized, we investigated the development of primary and secondary sex characteristics and measured gonadotropin and testosterone levels in 45 male adolescents with Down syndrome reared at home.[20] Pubic hair development did not differ significantly from that of normal boys. As is true for normal adolescent males, males with Down syndrome initially have darkening of the villous hair at the base of the penis; hair growth is then observed at the inguinal regions, the mons pubis, and the adjacent portion of the lower abdominal wall, later extending to the umbilical area.

Genital size of these patients was contrasted with that of an age-appropriate normal population. Mean testicular volume and penile length and circumference were not significantly different from the normal population at any age.

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels attained in a single morning sample were increased with advancing age and with sexual maturation, as shown in Table 2. No significant differences were found in those hormone levels when compared with normative data. Other investigators, however, have reported that serum FSH and LH levels were significantly higher in males with Down syndrome when compared with controls.[21-23]

Because most of the subjects in these older studies were residents in state institutions and were much older than the individuals in our study, it is possible that the observed testicular failure, including germinal cell hypoplasia and decreased Leydig cell function, was

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Table 1. Thyroid function studies in patients with Down syndrome and controls, mean ± SD

Group	N	T4 μg/dL	FT4 ng/dL	T3 ng/dL	FT3 pg/dL	T3U %	TSH μIU/mL	TBG μg/dL
Down syndrome	181	8.0±2.2	1.5±0.4	145.5±32.7	2.9±0.8	31.3±2.4	6.9±14	27.7±11.5
Controls	163	8.7±2.3	1.6±0.4	128.2±37.5	2.6±1.1	31.2±4.4	2.8± 7.8	29.4± 9.6
P value		0.003	0.09	0.0001	0.08	0.84	0.0014	0.14

T4, serum thyroxine; FT4, free thyroxine; T3, triiodothyronine; FT3, free triiodothyronine; T3U, triiodothyronine uptake; TSH, thyroid-stimulating hormone; TBG, thyroxine-binding globulin.

Table 2. FSH, LH, and testosterone levels in male adolescents and young adults with Down syndrome according to Tanner Genital Stages*

Tanner Stage	FSH, mIU/mL			LH, mIU/mL			Testosterone, ng/mL		
	Mean±SD	Range	Normal Range	Mean±SD	Range	Normal Range	Mean±SD	Range	Normal Range
1 (n=4)	3.2±3.4	1.0-9.0	3-9	4.3±1.7	2.0-6.3	4-12	0.4±0.2	0.1-0.6	0.03-0.1
2 (n=7)	2.2±0.5	1.8-2.9	3-14	3.7±0.5	3.2-4.4	6-11	0.9±1.0	0.1-2.4	0.1-0.3
3 (n=8)	7.0±3.7	2.2-14.0	3-15	9.3±2.9	5.7-14.9	6-16	3.4±1.8	0.8-5.6	0.7-4.0
4 (n=11)	10.5±7.8	3.9-30.0	4-15	15.5±9.0	4.3-33.0	7-19	5.7±1.3	2.9-7.4	2.5-9.0
5 (n=12)	8.6±4.6	2.7-19.9	4-13	11.4±5.0	5.3-21.6	6-23	4.7±1.9	1.3-8.3	3.5-12.0

FSH, follicle-stimulating hormone; LH, luteinizing hormone.
*Tanner stages are according to a previous study.

responsible for their findings. These discrepancies between our data and others' should prompt further investigation.

Fertility by males with Down syndrome is exceedingly rare. A recent report is the first of a nonmosaic male with Down syndrome conceiving a child with normal karyotype.[24]

Young females with Down syndrome have menarche at a mean age of 12 years 6 months, an age not significantly different from the menarche of their sisters who did not have Down syndrome.[24] Of the 38 females with Down syndrome who had menstruated at least once, 29 reported regularly occurring menses. The nine females with irregular cycles included three who had menarche only recently, two who had spotted several times without an established pattern, and four who had very irregular patterns but a normal to heavy flow. The average length of the monthly cycle varied from 22 to 33 days, with average menstrual flow lasting about 4 days. Thus, most young women with Down syndrome living in the community have regular menstrual cycles with age of onset similar to that of the normal female population.[25]

Our investigations of pituitary and ovarian hormones revealed that adolescent females with Down syndrome have concentrations of FSH, LH, and estradiol similar to those of a control population. The rise of FSH and LH during sexual maturation that is observed in individuals without Down syndrome also was observed in our population.

An investigation of follicular development in ovaries of females with Down syndrome was conducted by Hojager and coworkers.[26] They reported that all ovaries from patients with Down syndrome were abnormal, that 42% of their ovaries were quiescent with small, resting follicles and no follicular growth, and that the number, as well as the size, of the antral follicles differed from those in the normal ovary. In another study, Tricomi and coinvestigators[27] examined the ovulatory patterns in vaginal smears of females with Down syndrome. They found that nearly 40% of these women had a definite pattern of ovulation, another 15% probably ovulated, and another 15% possibly ovulated. There was no evidence of ovulation in the remaining 30% of females.

Pogue[28] reported in 1917 that women with Down syndrome are capable of reproduction. Since then, 30 pregnancies occurring in 26 women with Down syndrome have been reported.[25] Approximately half of these children were normal and half had Down syndrome. These reports suggest adequate ovarian function in at least some females with Down syndrome.

In summary, although some endocrine studies have been performed in individuals with Down syndrome, much remains to be done. The relationship between thyroid dysfunction and Down syndrome needs to be elucidated further. Although pubertal development appears normal, the overall potential for reproductive capability remains marginal; conception and childbearing in some females certainly does occur. Stature is usually reduced. Sophisticated studies of growth hormone, LH, and FSH pulsatility have not been reported; thus, little is known about the physiology of pituitary hormone secretion in these individuals.

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Inflammatory Bowel Disease and Growth Retardation

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Severe impairment of linear growth is a well-known complication of chronic inflammatory bowel disease (IBD) in childhood. Growth failure is often the major complication for which treatment is sought by the chronic IBD patient, and restitution of normal growth often signals remission of disease. Of all the manifestations of IBD in childhood, none is as poorly understood or as resistant to therapy as chronic growth failure. However, reversal of growth failure can be achieved by purely nutritional means.[1]

Growth Failure

Growth failure in children with IBD is a common and ominous complication (Figure 1). Impairment of linear growth, lack of weight gain, retarded bone development, and delayed onset of sexual maturation are seen in 15% to 40% of patients with IBD under 21 years of age.

Growth failure may precede clinical illness, often by years. Furthermore, growth failure may occur when clinical disease is quiescent. Under these circumstances, it must be assumed that the chronic demands placed on the body by the presence of undiagnosed inflammatory disease accounts for alterations leading to poor growth. Growth failure is rarely if ever associated with endocrine abnormalities. Tests of hormonal function generally are normal. Recent reports have demonstrated that some chronic IBD children with growth failure have low serum somatomedin-C levels.[2] However, somatomedins are dependent on protein intake, and serum levels rise after repletion of protein nutriture. Thus, this potential mediator requires further study and should not be identified as the final common pathway for growth failure in IBD.

Etiology of Malnutrition and Growth Failure

The etiology of malnutrition in patients with IBD is multifactorial and generally cannot be ascribed to a single event.[1] The major factors include inadequate dietary intake, excessive gastrointestinal losses, malabsorption, and increased nutritional requirements (Table 1). Inadequate dietary intakes in patients with IBD may occur as a result of the anorexia associated with chronic illness or recurrent bouts of inflammatory activity. Often children refuse to eat because of increased diarrhea or abdominal pain associated with the ingestion of food. Excessive losses of nutrients may originate from the gastrointestinal tract or through the kidneys. Large dosages of exogenous corticosteroids or the endogenous stress-induced response to acute inflammation may lead to increased urinary nutrient losses. Hematochezia, protein-losing enteropathy, and increased fecal losses of cellular constituents are consequences of chronic inflammation and damage to the in-

testinal mucosa. Bile salt-losing enteropathy and subsequent fat malabsorption result from ileal disease, resection, or fistulas.

Malabsorption is more common in patients with Crohn's disease, particularly individuals with small bowel involvement, and less common in ulcerative colitis. Hypoalbuminemia is found in at least 50% of patients due to undernutrition and/or increased fecal protein loss. Approximately 16% of IBD patients will have abnormal xylose absorption tests, whereas 33% will have a moderate degree of steatorrhea and increased bile acid malabsorption in conjunction with mucosal injury and bacterial overgrowth. Lactose intolerance may also be present either due to the presence of small bowel Crohn's disease or to the genetic background of the patient. Hypocalcemia and hypomagnesemia, when present, are generally associated with enteric protein loss or steatorrhea. Vitamin D deficiency has been described in 25% of older patients evaluated for bone disease associated with Crohn's disease. Vitamin K deficiency, when it occurs, is usually a consequence of steatorrhea. Reductions in serum iron and folate levels are common, and in severe ileal disease or resection, vitamin B_{12} deficiency is inevitable. Some children with Crohn's disease have reduced serum zinc levels, but the role of this trace element in conjunction with malnutrition and growth failure is unclear.

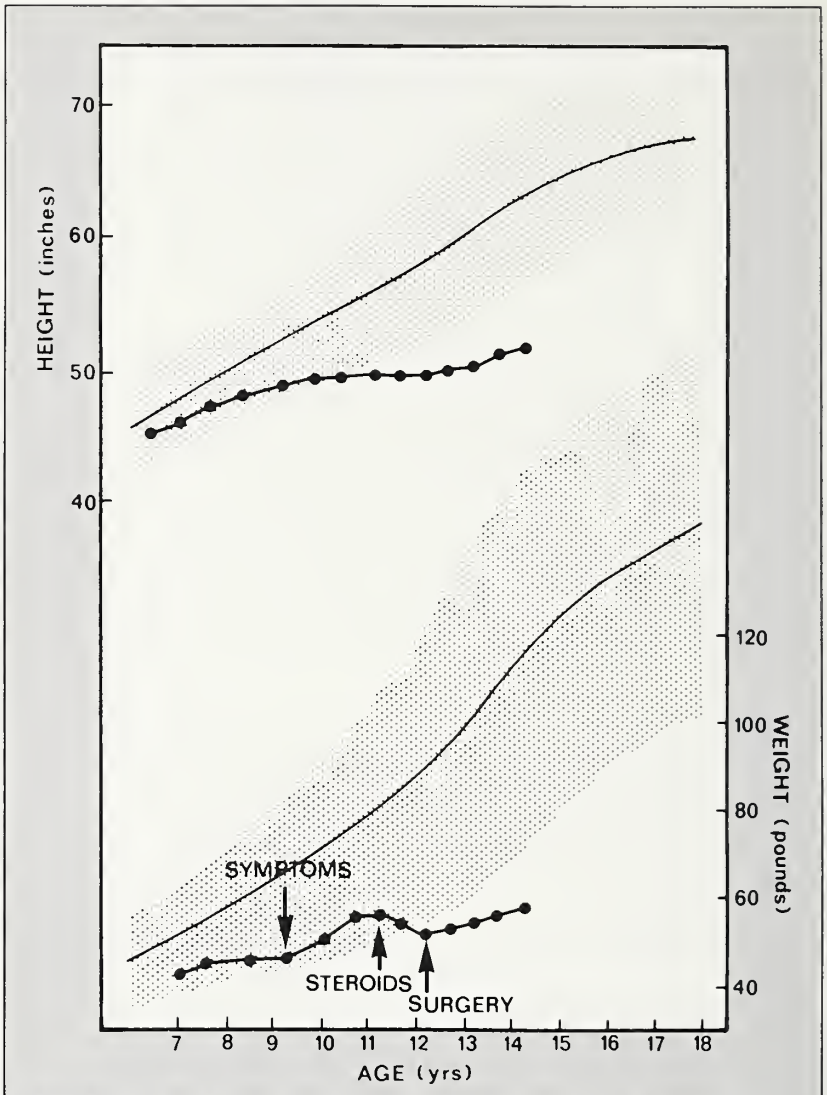

Figure An example of the growth pattern in a patient with Crohn's disease without nutritional intervention. The patient was in the 35th percentile for height and 25th percentile for weight prior to the onset of illness. Growth failure began prior to the onset of clinical symptoms. Subsequently severe impairment of linear growth and weight occurred that was not significantly improved by steroids or surgery.

Increased nutritional requirements may result from increased inflammatory activity, fever, intestinal fistulas, or periods of rapid growth, particularly during adolescence. Inflammation leads to negative energy and nitrogen balances as a result of decreased dietary intake and increased metabolic activity. Additional nutrient requirements are also a consequence of the demands on growth in children. With a peak weight gain of 7 kg per 6-month interval during puberty and at an energy cost of up to 4.4 cal/g of tissue gained, an additional energy intake of 170 kcal/day may be needed during the adolescent growth spurt.

Nutritional Assessment

Regular evaluations are necessary to assess the initial impact of nutritional failure on the child with IBD and growth failure, and to measure the success of therapy over time. Recommendations for nutritional assessment are shown in Table 2. It should be stressed that the use of this sequential assessment allows the clinician to maintain close surveillance not only over nutritional status but also over measurements of linear and ponderal growth. Alterations in therapy must be made in order to achieve and maintain normal expected growth rates. Carefully maintained data regarding growth and nutrition are mainstays of treatment of children with growth failure and IBD.

Treatment of Growth Failure

Medical. In the routine management of IBD, with or without growth failure, control of inflammatory activity is the first goal of medical treatment. Medications currently used for children with IBD are listed in Table 3, and discussed in detail elsewhere.[1,3] Sulfasalazine

Table 1. Etiology of malnutrition in IBD

Inadequate intake	**Excessive intestinal losses**
Anorexia	Protein-losing enteropathy
Altered taste	Hematochezia
Abdominal pain	Bile salt-losing enteropathy
Diarrhea	
Early satiety	
Malabsorption	**Increased requirements**
Protein	Fever
Carbohydrate (xylose, lactose)	Fistulas
	Repletion of body stores
Minerals (Ca, Mg, Fe, Zn)	Growth
Vitamins (folate, B_{12}, D, K)	
Bacterial overgrowth	
Drug inhibition (folate)	

Table 2. Evaluation of the nutritional status of children with IBD

History
- Appetite, extracurricular activity
- Type and duration of IBD, frequency of relapse
- Severity and extent of current symptoms*
- Medications

Three-day diet record

Physical examination
- Height, weight, arm circumference, triceps skinfold measurements
- Loss of subcutaneous fat, muscle wasting, edema, pallor, skin rash, hepatomegaly

Laboratory tests
- CBC and differential, reticulocyte and platelet count, sedimentation rate, urinalysis
- Stool guaiac, cultures for bacteria, smears for ova, parasites, and fat
- Serum total proteins, albumin, transferrin, retinol binding protein, orosomucoid, immunoglobulins
- Serum electrolytes, calcium, magnesium, phosphate, iron, zinc
- Serum folate, vitamins A, E, D, B_{12}

Special tests
- Xylose absorption, 72-hour fecal fat, fecal α-1-antitrypsin, lactose breath test, Schilling test

Radiology
- Upper GI series with small bowel follow-through
- Air-contrast barium enema

Colonoscopy with biopsies

*Crohn's Disease Activity Index (*Gastroenterology* 1976;70:439) or Lloyd Still Clinical Scoring System (*Dig Dis Sci* 1979;24:620) may be useful in the assessment.

Table 3. Commonly used drugs in treatment of IBD

Drug	Daily Dose	Comment
Sulfasalazine	50 mg/kg	May increase to 75 mg/kg or standard adult dose
Steroids (prednisone, prednisolone)	1-2 mg/kg	Single AM dose when possible Dose depends upon severity Not to exceed standard adult dose
Azathioprine or 6-MP	2 mg/kg 1.5 mg/kg	Not to exceed standard adult dose
Metronidazole	15-20 mg/kg	Not to exceed 1.0 g

is recommended for the treatment of mild acute attacks and maintenance of remission when the colon is involved. Some patients with small bowel Crohn's disease will also respond to sulfasalazine therapy, but less predictably.

In contrast, prednisone is more effective in treating moderate to severe activity of disease. Corticosteroids induce remissions, but do not prevent relapses, and may, in fact, increase overall morbidity when used as maintenance therapy. Therefore, corticosteroids are generally recommended in limited courses, using a single morning dose when the severity of the disease permits this form of therapy. Sometimes, twice daily or more frequent oral doses are necessary. Therapy should be maintained for 4 to 6 weeks, tapering to an alternate-day regimen by decreasing the dosage by 5 mg every other day at 5- to 7-day intervals. If necessary, prolonged alternate-day therapy may be maintained. In most cases, this regimen allows for a gradual decrease of medication without flare-up of disease. Low-dose, alternate-day steroid therapy is an acceptable form of long-term treatment.

Many patients with IBD demonstrate accelerated linear growth, despite high-dose steroid therapy, presumably because inflammatory activity is suppressed.[3] An improvement in appetite may account in part for the growth response, because increased dietary protein and energy intakes are associated with corticosteroid use. This may be particularly true when alternate-day steroid therapy is used for a prolonged period of time.

Other medications may be valuable in bringing disease activity under control. Azathioprine and 6-mercaptopurine may allow reduction in the dosage of steroids required, prolong remission, avoid surgery, and allow prolonged maintenance in patients who would not be candidates for other forms of therapy. Metronidazole is valuable for perianal disease, and this agent or vancomycin may be

helpful in those patients whose flare-up of disease activity is associated with *Clostridium difficile* overgrowth.[3]

Surgical. Surgical resection of disease has been considered as an alternative in the management of growth failure in patients with IBD, but the results of this approach have not supported its routine use for this purpose. In most studies, children with Crohn's ileocolitis have only limited response to removal of active disease, with only 14% to 28% of patients showing postoperative catch-up growth. Virtually all children who have had catch-up growth after surgery were prepubertal at the time of operation. In general, pubertal patients have shown no catch-up growth after surgery. At the present time, bowel resection should be reserved for those patients in whom there is another clear indication for surgery besides growth failure. In selected cases, where medical and nutritional therapy have failed to alter growth arrest, surgical treatment may be beneficial in prepubertal children.[3]

Nutritional. Even in the absence of nutritional failure or growth retardation, the indications (Table 4) and benefits of nutritional therapy in IBD have become apparent. In the nutritional management of children with growth failure and IBD, the major aims are to replace the nutrient losses that are associated with inflammatory processes, to correct body deficits, and to provide sufficient nutrients to promote energy and nitrogen balance for normal metabolic function. In children, additional nutrients must be provided to restore normal growth and to provide catch-up growth.[1]

Both enteral and parenteral routes are available for the treatment of nutritional disorders in IBD (Table 5). The easiest way to provide nutritional supplementation is to increase intake enterally, using standard table foods. No specific diet has been shown to alter the course of ulcerative colitis or Crohn's disease in patients who are in remission. There is also no clear evidence that the consumption or avoidance of specific foods influences the severity of disease or the frequency of relapses, or induces remission. Accordingly, patients should be encouraged to eat an adequate, well-balanced diet and to avoid food fads. In children and adolescents, it is preferable to allow the intake of favorite foods and beverages rather than force a limited energy intake.

When disease is active, when specific foods exacerbate symptoms, or when laboratory tests suggest specific abnormalities such as steatorrhea or lactose intolerance, the diet should be appropriately modified. In the presence of postprandial pain, a low-residue diet, administered as frequent small meals, is often recommended. In children with watery diarrhea due to bile acid or hydroxy fatty acid excretion, a low-fat diet supplemented with medium chain triglycerides and the use of cholestyramine may be helpful in the control of symptoms. However, care must be taken to ensure that patients on low-fat diets are consuming adequate energy intakes.

Table 4. Indications for nutritional therapy in IBD

Primary therapy for disease activity
Newly diagnosed IBD
Chronic disease unresponsive to medical management
Short bowel syndrome
Closure of fistulas
Small bowel obstruction
Ostomy care
Supportive therapy for disease activity
Inoperable diffuse disease
Preoperative nutritional rehabilitation
Drug-nutrient interactions
Sulfasalazine (folic acid)
Abnormalities of specific laboratory test
Anemia (microcytic, macrocytic)
Hypoproteinemia
Fat malabsorption
Lactose intolerance
Serum mineral deficiencies (Fe, Ca, Mg, K)
Serum vitamin deficiencies (folate, B_{12}, A, D)
Prolonged prothrombin time (vitamin K)
Depressed alkaline phosphatase (Zn)
Complications of IBD
Malnutrition
Growth failure

Table 5. Nutritional therapy of IBD

Well-balanced, high-protein and -energy diet	
± Low residue	
± Lactose-free	
± Low fat; MCT and cholestyramine supplemented	
Enteral supplementation	
(140% to 150% of Recommended Daily Allowances for height age)	
Continuous intermittent nasogastric tube feeding	
Continuous or intermittent feeding gastrostomy	
Total parenteral nutrition	
(140% to 150% of Recommended Daily Allowances for height age)	
Peripheral	
Central	
Minerals and Vitamins	
Therapeutic	
Iron	
Ferrous sulfate (20% Fe)	6 mg elemental Fe/kg/day,
Ferrous gluconate (11.5% Fe)	divided in 3 oral doses
Iron dextran (intramuscular) (Imferon)	Follow directions on package insert
Magnesium	200-400 mg elemental Mg/day, IV
Zinc sulfate (22% Zn)	50-100 mg elemental Zn/day, divided in 3 oral doses
Vitamin B_{12}	1,000 mg at 3-month intervals, SC or IM
Folate	1 mg daily
Supplemental	
Multivitamins with minerals (daily)	

Table 6. Effect of nutritional supplementation on growth in adolescents with IBD

Measurements*	Control (n = 5)	Crohn's Disease (n = 6) Before Supplement	Crohn's Disease (n = 6) After Supplement
Observation period (mo)	6.5 ± 0.2	10 ± 1.4	7 ± 0.8
Height gain† (cm/mo)	0.38 ± 0.12	0.1 ± 0.08	0.5 ± 0.16§
Weight gain‡ (kg/mo)	0.4 ± 0.17	0.21 ± 0.09	1.22 ± 0.25§,‖

*Values are mean ± SEM; data are derived from Motil et al, *J Pediatr* 1982;101:345-351
†Expected linear rate for age, 0.47 cm/mo
‡Expected weight gain for age, 0.5 kg/mo
§$P<0.05$ vs before supplementation
‖$P<0.01$ vs control

Multivitamins with minerals should be administered routinely to replace deficits in the diet. Oral iron and folic acid therapy should be provided when laboratory findings are consistent with a deficiency state. Parenteral administration of vitamin B_{12} may be necessary in patients with extensive ileal resection. Despite an association between serum zinc levels and linear growth delay, very few patients with growth failure have low serum zinc levels. However, those who have this abnormality are generally treated with oral zinc supplements.

When the patient is unable to increase dietary protein and energy intakes with larger meals or palatable snacks, oral supplementation with a commercially available liquid formula should be attempted. Successful supplementation may be achieved with such formulas; however, many patients experience early satiety when taking these, and will not increase their total nutrient intake significantly. Under these circumstances nutritional supplementation can be accomplished by intragastric feedings or parenteral alimentation.

Nasogastric infusions, used either continuously or intermittently, have been effective in reversing metabolic imbalances and improving nutritional status, linear and ponderal growth rates, and the clinical well-being of patients with IBD.[4-10] If the patient does not tolerate this form of therapy, a gastrostomy may be performed for either continuous or intermittent tube feedings in the same manner as in the nasogastric regimen. The gastrostomy tube is advantageous because it is cosmetically acceptable and easily cared for, and because large increases in the amount of formula can be administered in spite of the patient's lack of appetite. In our experience the only complication associated with intragastric tube feedings has been reversible diarrhea secondary to overly rapid administration of the nutritional supplement.

The amount of supplementation administered via the nasogastric or gastrostomy tube will vary, depending on the nutritional requirements and tolerance level of the individual. In our adolescent patients, up to 1,500 mL of a commercial formula, administered nightly for 8 to 10 hours, is usually well tolerated. This volume of supplemental formula, in addition to usual meals and snacks, provides protein intake of 3 g/kg/day and energy intake of 95 kcal/kg/day. Results of supplementation are shown in Table 6. After 3 weeks of nutritional supplementation, a weight gain of as much as 4 kg may occur, nitrogen balance improves, and total body potassium increases significantly. After 7 months of nutritional supplementation, average height and weight velocities were at least five times greater than those observed during the 10 months prior to supplementation, and equaled or exceeded velocities of normal adolescents.

These observations demonstrate that the abnormalities in the nutritional status of adolescents with Crohn's disease, malnutrition, and growth failure are not related to intrinsic defects in their metabolic pathways, and that with appropriate nutritional supplementation, growth occurs. Moreover, in these patients, neither the presence of chronic inflammation nor the use of corticosteroids interfered with their rehabilitation.[11,12] We also have recommended that commercially prepared formulas

In Future Issues

IGF-Binding Proteins: Their Physiological and Clinical Importance
by Michael Ranke, M.D.

Genomic Imprinting
by Judith G. Hall, M.D.

How Bones Grow
by William A. Horton, M.D.

Paracrine Aspects of Bone Metabolism
by David Baylink, M.D.

Complications of Excessive GH in Acromegaly
by Mark Hartman, M.D.

Imaging of the Endocrine System
by Andrew Poznanski, M.D.

Robinow Syndrome: An Update
by Meinhard Robinow, M.D.

Childhood Obesity
by William Dietz, M.D.

be used as an adjunct rather than as the sole source of long-term nutritional intake in order to avoid potential nutrient imbalances.

When patients with IBD are unable to tolerate adequate amounts of enteral alimentation because of disease activity or diarrhea, parenteral alimentation may provide substantial benefits. Parenteral nutrition appropriately improves nutritional status as demonstrated by linear and ponderal growth rates, lean body mass deposition, and postoperative recovery.[5] Nutritional rehabilitation may also induce a clinical remission.[1] Home parenteral alimentation is available for those patients who require long-term nutritional support for active disease, short bowel syndrome, or growth failure.[13] In general, the nutritional recommendations are similar to those used for enteral nutrition support.[1] Patients may be monitored by their own hospital programs or by a commercial nutritional maintenance company.

Conclusions

Early nutritional intervention is essential in the management of chronic IBD. Individual nutrient deficiencies may occur in children, but more frequently there is a generalized protein-energy malnutrition complicated by progressive growth retardation. The etiology of malnutrition in disease is multifactorial. Patients at risk for developing malnutrition or its complications are those individuals with long-standing disease and weight-for-age deficits. Nutritional intervention provides support during active inflammatory disease, treatment of individual deficiencies, reversal of malnutrition, and stimulation of growth. Prevention of nutritional disorders and their complications in IBD is possible by carefully monitoring appropriate anthropometrics and laboratory indices, and by promptly instituting enteral or parenteral nutrition rehabilitation as soon as indicated.

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Commentary: Abortion, Politics, and Science

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Of the inheritable diseases, Lesch-Nyhan syndrome is one of the most devastating. Affected boys are not only profoundly mentally retarded, they engage in compulsive self-mutilating behavior, chewing on their fingers, shoulders, lips—whatever they can reach. Lesch-Nyhan syndrome is especially frustrating because, unlike many inherited illnesses, we understand its pathogenesis—the sex-linked disorder is caused by lack of hypoxanthine phosphoribosyl transferase—but there is little we can do to help these children. Current efforts at gene therapy to correct the enzyme deficiency have shown promise in vitro, but have yet to solve the probiem of how to deliver the enzyme (or its gene) to the appropriate brain cells and induce activity or expression at the appropriate time in development.

Lacking treatment, families with the Lesch-Nyhan gene often seek to have daughters, each of whom stands a 50% chance of being a carrier like her mother, and a 50% chance of being neither carrier nor affected. In contrast, each son has a 50% chance of having the illness. To increase their chance of having a daughter, parents can seek to avail themselves of one of three methods.

Artificial insemination with enrichment for X-bearing sperm can shift the odds somewhat in favor of a girl. Alternatively, parents can turn to in vitro fertilization (IVF). With DNA amplification via the polymerase chain reaction, the DNA of a single blastomere each from several zygotes can be amplified and exposed to Y-specific DNA probes, in an attempt to identify the Y chromosome. If a female zygote is identified, it can then be transferred to the woman's uterus by IVF.

However, until recently, the usual route followed by most parents was simply to abort any male fetuses identified by chorionic villus sampling or amniocentesis. Of course, Lesch-Nyhan syndrome was not the only sex-linked disorder in which prenatal diagnosis ended in abortions of healthy male fetuses half the time; pregnancies involving Duchenne muscular dystrophy or hemophilia presented the same dilemma.

Fortunately, progress has been made in several sex-linked disorders, including Lesch-Nyhan, so that parents may no longer have to

face the terrible choice of aborting potentially healthy males. DNA amplification and polymerase chain reaction techniques used in the first trimester can now help distinguish between Lesch-Nyhan-affected and unaffected males. The next frontier is to push diagnosis back even further to the pre-embryo stage, and thus preclude many of the emotional issues involved in abortion.

Pre-embryos, tiny spheres whose cells have not yet even "decided" whether they are to become part of the embryo proper or of an extra-embryonic membrane, can be manipulated to reveal information of great benefit to families with sex-linked disorders. Moreover, understanding the metabolic requirements of pre-embryos during those crucial first few days may explain why IVF fails far more often than it succeeds; why millions of zygotes conceived the conventional way do not implant into the endometrium properly; why for every 100 conceptions only 31 survive to be born. We still know comparatively little about this period of the zygote. This is a time in development that has remained shielded from the increasingly commonplace tools of obstetrics; eg, at-home pregnancy kits, ultrasound, chorionic villus sampling and amniocentesis, and alpha-fetoprotein testing.

These are compelling questions, whose answers are not likely to be discovered in the United States. Due to the many ethical/legal issues, research on pre-embryos, embryos, and fetuses is currently blocked by the US government's disregard for federal regulations that in fact provide a mechanism for consideration of such projects. Since 1980, the federal government has made progress in this field virtually impossible because abortion politics, rather than scientific considerations, dominate policy concerning the prenatal human. The result is a bizarre "catch-22" situation: Federal regulations governing human subjects for research ban any federal funding of projects involving the pre-embryo and sharply restrict any project entailing more than "minimal risk" to the fetus—unless a Health and Human Services (HHS) Ethical Advisory Board (EAB) recommends a waiver of the rules.

But in 1980, HHS officials, fearing political controversy, let the EAB be disbanded—leaving researchers in the incredible predicament of being obliged by law to have their project approved by an entity that does not exist. Outgoing HHS Secretary Otis Bowen did not approve the charter of a new EAB before leaving office. Thus, for the past 9 years, HHS has been in violation of its own regulations.

The United States' reticence toward exploring the biology of the unborn extends to the fetus as well. In May 1988, HHS imposed a moratorium on federally supported human fetal tissue transplant research aimed at relieving conditions such as Parkinson's disease and juvenile diabetes. In early January 1989, an advisory panel appointed by the National Institutes of Health (NIH) recommended that funding be restored. But despite the NIH report, the moratorium has now become a ban on federal funding for research with fetal tissue obtained after induced abortion. This ban was extended by Assistant Secretary for Health James O. Mason.

Meanwhile, talented US researchers are being forced to put on hold or abandon their ideas, or to seek private funding. Consider Oliver H. Lowry, a Washington University biochemist whose experimental protocol utilizing very early human zygotes received a high priority rating by NIH. He was subsequently denied funding because an EAB was not in place. Dr. Lowry, struggling to continue on short-term private funding, is investigating the metabolites and enzymes needed by, and hazardous to, fertilized human ova and early zygotes developing in vitro. Says Dr. Lowry, "The advent of IVF has raised a problem and created an opportunity. The problem is that the abnormal fertilized ova that are now discarded could be used for scientific study. With modern techniques, these discarded ova could provide answers to what may be wrong with present in vitro procedures. But even more importantly, such studies could give clues to the cause of early pregnancy loss and fetal malformations in general."

In Great Britain in 1982 a committee of inquiry of the Department of Health and Social Security was convened. It was chaired by Mary Warnock, a philosopher, and consisted of scientists, social workers, lawyers, ethicists, and health administrators. Unlike many other groups charged with answering the eternal question, "When does life begin?" this one actually set some boundaries, concluding, "A human embryo cannot be thought of as a person, or even as a potential person. It is simply a collection of cells, which, unless it implants in a human uterine environment, has no potential for development." Although implantation generally occurs on days 5 to 7 post-fertilization, the committee set day 14 as the time before which research would be permitted. The reason for this cut-off point is anatomical: day 14 is the time of appearance of the primitive streak, the first rudimentary inkling of a central nervous system. This certainly contrasts with the position of a Maryville, Tennessee, judge, who recently bestowed upon the frozen zygotes of a divorced couple the status of "children."

In West Germany, the sort of work being done in Great Britain would be a criminal offense punishable by up to 5 years in prison, if a law drafted in July 1989 is passed in early 1990, as is expected. The country's two main financial providers for scientific research have vowed to refrain from funding embryo work. Behind the Germans' restraint lie several factors: memories of the horrors of Nazi eugenic experiments, the volatile German politics of today, and Chancellor Kohl's need to show that he hears the opposition of the Green Party and religious groups to this research.

No one wants to see women intentionally becoming pregnant in order to sell harvested fetal parts. Every type of research involving human embryos or fetuses requires and should always require approval by appropriate committees. The British, for example, forbid research fusing a human cell with a nonhuman cell and allowing a chimera to develop, or implanting a human fertilized ovum or zygote in the uterus of a nonhuman. Both of these cross-species experiments have been done in nonhuman mammals.

The potential scientific and health benefits of research involving the embryo (and the fetus under some conditions) are now threatened by the debate over the morality of abortion and the nature of the embryo. Without a sensible and coherent national policy on fetal research, infertile couples, families at high genetic risk, fetuses with disorders that may be correctable, and patients afflicted with brain disorders will continue to suffer. So will scientific knowledge.

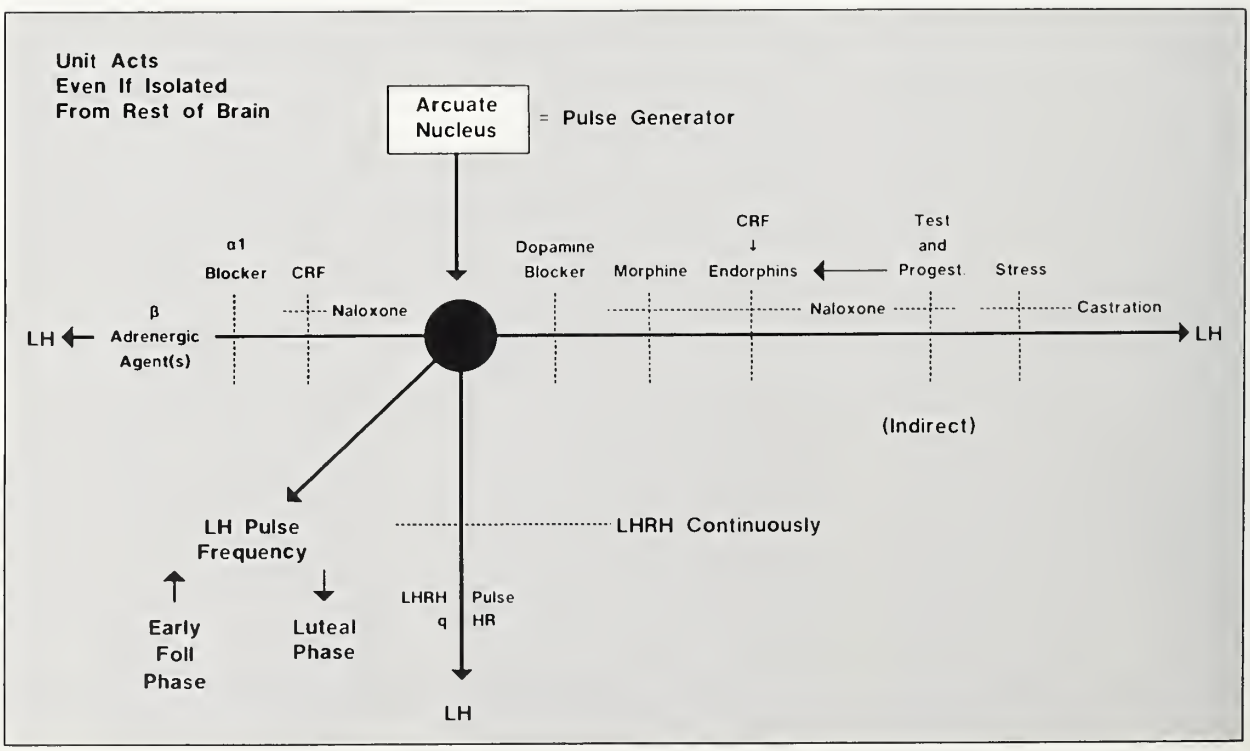
Special Report

Third International Conference on the Control of the Onset of Puberty, May 7-10, 1989, Amsterdam

Robert M. Blizzard, M.D.
Chairman
Growth, Genetics, and Hormones

The last International Conference on Puberty was held in 1981. In the intervening 8 years much emphasis in investigation has been placed on molecular biology. The content of this 4-day meeting was approximately 80% basic physiology or microbiology and 20% clinical investigation of the onset of human puberty. The proceedings of this conference, which should be available by the time this issue of *Growth, Genetics, and Hormones* arrives on your desk, will provide a valuable resource for all basic scientists and endocrinologists who have an interest in the physiology and pathophysiology of adolescence.

A highlight of the meeting was an outstanding review by Professor Ernest Knobil of the past and present knowledge regarding luteinizing hormone (LH) pulsatility in the primate. Professor Knobil emphasized that the gonadotropin-releasing hormone (GnRH) pulse generator continues to work when the arcuate nucleus-pituitary



axis is isolated from the rest of the brain in vivo by surgery and that destruction of the arcuate nucleus in the intact monkey negates the pulse generator. Electrodes in the arcuate nucleus block only the release of LH and FSH; the production and secretion of other pituitary hormones remain intact. Down-regulation of LH receptors occurs with constant infusion of luteinizing hormone releasing hormone (LHRH), but if pulses of LHRH are given every hour, ovulatory cycles are induced. This establishes the physiological necessity for pulses of LHRH to occur. Estrogens do not seem to affect the pulse frequency of LH, as both postmenopausal women and younger women during the midfollicular (estrogen) phase of the cycle have the same frequency of pulsations. The acceleration of the pulse generator during the early follicular phase may be attributed to a release from the inhibitory action of progesterone. Knobil discussed the roles played by neurotransmitters and hormones in the release of LH, and these roles are graphed for easier assimilation (Figure).

Dr. T. Plant of Pittsburgh presented a scholarly paper regarding the ontogeny of GnRH secretion in the rhesus monkey. The differences in basal LH and FSH and in the time of onset of pulsatile LH and FSH between males and females were discussed. The explanation of these differences remains obscure. A fascinating part of Plant's presentation dealt with the administration of estrogen, testosterone, and dihydrotestosterone to female and male prepubertal monkeys. All stimulated body weight and length gain, but only the first two accelerated skeletal maturation. Plant conjectured that this may be because dihydrotestosterone is not aromatizable.

There were many other excellent presentations and readers may wish to consult the proceedings, which are published in a supplement to *Acta Scandinavia*.

Special Report

The 4th National Cooperative Growth Study Conference
November 18-21, 1989, Palm Springs, California

Robert M. Blizzard, M.D.
Chairman
Growth, Genetics, and Hormones

Among the most important topics covered at this conference were those that pertained to growth hormone (GH), insulin-like growth factor (IGF) binding proteins, and related receptors. The GH receptor is found in large quantities in rabbit liver but also in kidney, muscle, bone, and brain (hypothalamus). Its gene is on the short arm of chromosome 5. The receptor is one of a family of a new type of receptors (prolactin and GH) that serves as a binding protein in plasma and as a receptor in tissue. These receptors do not act through tyrosine kinase and are unrelated in amino acid sequence to other known receptors. The plasma binding protein (BP) is probably the external portion of the GH receptor, which extends outside the cell membrane. This BP and the receptor have both been reported to be absent in Laron dwarfism. In order to analyze the receptor gene in patients with Laron dwarfism, nine patients with this entity were studied; two had a deletion of a large portion of the extracellular hormone-binding domain of the receptor gene.

The IGF receptors (IGF-1 and IGF-2) and the insulin receptor are frequently considered together, because the receptors for insulin and IGF-1 are closely related and bind IGF-1, IGF-2, and insulin in various proportions. The receptor for IGF-2, which is also the mannose phosphate receptor, is of a completely different structure and binds IGF-1 and IGF-2 but not insulin. IGF-2 promotes growth by acting through the IGF-1 receptor. IGF-2 *does not* promote growth through its interaction with the IGF-2 receptor, and what role that interaction does play remains obscure.

There are at least 3 IGF BPs. The major BP is BP-3, which comprises 98% of the circulating IGF BP and which is under GH control. IGF BP-3 increases and decreases concomitantly with GH production. This BP is produced in both breast and liver cancer as well as in intact liver. It is a large glycoprotein complex (140 kDa) that has a nonbinding alpha subunit (acid labile) and a binding beta subunit (acid stable). These two BP-3 subunits, along with IGF, which is bound to the beta subunit, comprise the large BP.

The three BPs may be responsible for the true autocrine functions of IGF-1 and IGF-2. Because of its binding characteristics for IGF-1, BP-3 may protect the individual against the hypoglycemic effect of IGF-1 and increase the half-life of IGF-1. In uremia this BP-3 is increased to very high levels, possibly because the kidney clears this protein. With chronic renal disease the marked increase in BP-3 may act as the "inhibitor" described for IGF-1 in kidney disease: The excess BP-3 may bind IGF-1 so there appears to be only a small amount of IGF-1 present, which is then misinterpreted as the presence of an inhibitor.

The presenters who addressed these issues were Dr. Michael Ranke of Tübingen, FRG, Dr. Ron Rosenfeld of Stanford University, Palo Alto, CA, and Dr. William Wood of Genentech, South San Francisco, CA.

Robert M. Blizzard, M.D.

Editor's note: *Dr. Ranke will be contributing a lead article titled "IGF BPs" in a forthcoming issue of* Growth, Genetics, and Hormones. *In it, Dr. Ranke will discuss how measuring IGF BP-3 may assist in diagnosing GH deficiency.*

Prenatal Diagnosis for Pediatricians: Committee on Genetics of the AAP

Rapid advances in technology have prompted this Committee to prepare a new report to inform pediatricians and others in the medical profession about the current status of antenatal diagnosis of genetic disorders and guidelines for parental counselling. The recommendations of the new report are as follows:

Fetal chromosome analysis should be offered when:
- maternal age is advanced.
- a previous offspring has a trisomy condition.
- a chromosome abnormality is present in a parent.
- the fetus is at risk for a serious X-linked condition and specific intrauterine diagnosis is unavailable.
- a parent is a "fragile X" carrier.
- a fetal abnormality (eg, omphalocele, hydrocephalus, etc.) has been identified by ultrasound, which might indicate an increased risk for karyotypic abnormalities.

Biochemical studies are indicated when:
- a previous child is affected with a biochemical condition.
- couples are at risk because of possible carrier status related to their ethnic origin (eg, sickle cell disease, Tay-Sachs disease).
- neural tube defects are present in a parent or sibling.
- couples are at increased risk for having an infant with a neural tube defect (eg, a low alpha-fetoprotein level is found in maternal serum at screening).

Molecular genetic studies are of potential benefit when:
- hemoglobinopathies, hemophilia-A, Duchenne or Becker muscular dystrophy, or cystic fibrosis may reasonably be suspected.

Techniques for tissue sampling, including discussion of amniocentesis, chorionic villus sampling, fetal blood sampling, fetal skin sampling, and organ biopsies are also discussed, as are the techniques for fetal visualization, such as ultrasound, fetoscopy, magnetic resonance imaging, and radiography.

A copy of the report should be kept readily available in every pediatrician's office and reviewed frequently.

Committee on Genetics of the AAP
Pediatrics 1989;89:741-744.

Robert M. Blizzard, M.D.

Biopsy of Human Preimplantation Embryos and Sexing by DNA Amplification

With improvement of in vitro fertilization techniques, major advances in handling preimplantation embryos, and the advent of the polymerase chain reaction (PCR), it was just a matter of time until nondestructive biopsy of a human embryo allowed diagnosis before implantation. In this report, single cells were removed from 38 human embryos at the 6-10 cell cleavage stage (3 days after in vitro fertilization). The individual blastomeres were then subject to amplification of a Y-specific repeat sequence through 60 cycles. On day 6 the embryos were analyzed cytogenetically to determine chromosomal sex using fluorescent Y chromosomes and in situ hybridization with Y-specific probes. Of the 15 "normal" embryos (ie, possessing two pronuclei), all were correctly sexed by means of DNA amplification. Results were available within 8 hours, which means a "normal," specific-sexed embryo could be transferred to the uterine environment the day of diagnosis. Biopsied embryos appeared to have normal development to the blastocyst stage in the same proportion as unmanipulated embryos, and good morphologic development to a mean of 35.6 cells occurred. M. Monk (*BioEssays* 1988;8:184-189) has previously reported similar techniques for the purpose of preimplantation diagnosis.

Handyside A, Penketh R, Winston R, et al. *Lancet* 1989;1:347-349.

Editor's comment—*This technique is readily applicable to any disorder for which the gene has been identified and PCR primers developed that can distinguish the mutant gene(s). Preimplantation diagnosis is technically possible and appears to be relatively safe for the embryo; however, more work concerning safety is needed in animal models. This technique is valuable because it allows the selective implantation of an XX karyotype embryo into the female of a couple at risk for male infants with an X-linked inherited disease such as hyperphosphaturic hyperphosphatemic rickets. Early work on a mouse model for Lesch Nyhan syndrome is very promising.*

Judith G. Hall, M.D.

Gross and Fine Motor Development in 45X and 47XXX Girls

Results of this study indicate that gross and fine motor developmental delays are associated with a mild to moderate sensory-motor integration dysfunction in 45X and 47XXX girls followed over many years. In contrast, near-normal functioning in these parameters was seen in 45X/46XX mosaic girls. The incidence of these chro-

Oocyte Donation as a Means of Achieving Pregnancy in Women with Primary or Secondary Ovarian Failure

Two groups providing oocyte donation for achieving pregnancy report promising results. A total of 77 patients (from both studies), including women with Turner syndrome, premature ovarian failure, or with a 46 XY karyotype, underwent oocyte donation. Pregnancy rates were in the range of 35% per cycle (rates similar to other forms of in vitro fertilization). Multiple pregnancies (twins/triplets) and preeclamptic toxemia were frequent complications, but all newborns were normal [one with intrauterine growth retardation (IUGR)] and the miscarriage rate was low. Nine women with Turner syndrome (with a variety of karyotypes) had successful pregnancies. Best results were achieved in women who were regularly cycling prior to attempting pregnancy.

Serhal PF, Craft IL. *Lancet* 1989;1: 1185-1187.

Hens L, Devroey L, Van Waesberghe M, et al. *Clin Genet* 1989; 36:81-91.

Editor's comment—*This is good news indeed for Turner syndrome women. It means they have options for reproduction "just like everyone else." The data do suggest, however, that regular monthly cycling prior to attempted pregnancy gives the best results, so appropriate monthly cycling is recommended for Turner women considering this option at any time in the future.*

Judith G. Hall, M.D.

mosome abnormalities in 20,000 consecutive apparent female infants was 1:1,000. The defects in gross motor function included running speed and agility, balance, bilateral coordination, strength, and upper limb coordination. Defects in fine motor function included abnormalities related to upper limb speed and dexterity, visual motor control, and speed of response. Walking late—between 15 and 22 months—was present in 9 of the 15 45X and 47XXX girls studied.

Of these 15, 11 manifested moderate to severe language dysfunction and 12 were referred independently for special educational services. The mean IQ for those in the 45X and 47XXX groups was 83.0, whereas the mean IQ for the mosaic group was 102.0, which was the same as for the normal control group.

The authors conclude that sex chromosome aneuploidy in girls is associated with an increased risk for sensory-motor integration dysfunction. This is likely to be an additional factor that negatively influences classroom performance along with the language delays and depressed cognitive abilities frequently found in these girls. Regular developmental assessments are recommended to provide anticipatory guidance through early identification and intervention. Neuromuscular status and sensory-motor integration should be ongoing as part of the evaluation of children with sex chromosome aneuploidy.

Salbenblatt JA, Meyers DC, Bender BG, et al. *Pediatrics* 1989; 84:678.

Editor's comment—*The authors have added significantly to our knowledge and understanding of the mental and physical performance of girls with sex chromosome aneuploidy. I, for one, will be more attentive to these frequent problems in such girls.*

Robert M. Blizzard, M.D.

Hyponatremia and Inappropriate Secretion of Vasopressin in Patients with Hypopituitarism

Significant and symptomatic hyponatremia is reported in five patients with hypopituitarism that included adrenocorticotropic hormone (ACTH) deficiency. These patients, although adults, had severe hyponatremia without significant dehydration. Vasopressin [antidiuretic hormone, (ADH)] levels were increased and believed to be the etiology of the inappropriate ADH syndrome. Glucocorticoids are believed to suppress vasopressin production and release, and all patients were restored to the normal osmolar state following physiological doses of hydrocortisone.

The author concludes that hypopituitarism (actually hypocortisolism) should be considered in the differential diagnosis of hyponatremia without dehydration because, given the correct diagnosis, treatment with glucocorticoids is much more effective than treatment with hypertonic saline. The author notes that it is possible to have low basal cortisol levels in this syndrome, and such levels should not preclude suspicion of its presence.

Oelkers W. *N Engl J Med* 1989; 321:492.

Editor's comment—*An abstract of this excellent article is appropriate because many pediatric endocrinologists are unaware that hyponatremia results from cortisol deficiency. Cortisol replacement is very much needed in patients with hypopituitarism that includes relative or actual ACTH deficiency. Cortisol replacement is highly desirable in such patients although, in dealing with children, we must be careful not to exceed physiological replacement, in order not to produce growth inhibition.*

Robert M. Blizzard, M.D.

Growth, Genetics, and Hormones Index for Volume 5, 1989

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The Biology of Bone Growth

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Linear bone growth is a very complex biologic process. Although much is known about the circulating factors that influence it, much less is understood about the process itself, especially in humans. Indeed, to a large extent it has been viewed as a "black box," which when appropriately stimulated generates longer bones. However, as non-growth hormone deficient types of growth deficiency, such as the chondrodysplasias, receive greater attention, it becomes necessary to dissect the black box and examine its mechanisms.

The genesis of the embryonic skeleton and its subsequent linear growth arise from the same three fundamental phenomena: chondrogenesis, cartilage hypertrophy, and osteogenesis. Chondrogenesis is responsible for the formation of a cartilage model of the skeleton and most of its subsequent physical lengthening. Hypertrophy contributes to some extent to lengthening, but its role is mainly to facilitate the transition of the cartilage model to bone. Osteogenesis produces the final skeletal form. After embryogenesis all three processes integrate in a smoothly functional unit that structurally corresponds to the growth plate. A closer examination of the process reveals how this occurs.

The current biologic model of skeletogenesis is derived from studies of limb development in lower vertebrates, especially the chick. Skeletogenesis begins early in embryogenesis, with the outgrowth of limb buds composed of mesenchymal tissue covered by a layer of ectoderm (Figure 1A). The extracellular matrix produced by the poorly differentiated mesenchymal cells contains noncartilaginous molecules such as types I and III collagen, fibronectin, and small proteoglycans. Soon after the bud is formed, in areas destined to become bone, these cells form cellular condensations coincident with the appearance of mRNAs for cartilage-specific proteins, including type II collagen and cartilage (large aggregating)

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Autocrine and Paracrine Aspects of Bone Metabolism

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One of the major functions of bone is to provide mechanical support to the body. The strength of bone depends on its volume, which in turn is determined by the balance between two opposing processes, osteoblastic bone formation and osteoclastic bone resorption. Faulty regulation of this balance leads to disease states, eg, osteoporosis, making studies of the mechanisms of this regulation essential.

Two mechanisms have been postulated for the maintenance of bone volume: (1) systemic regulation by calcium- and phosphate-regulating hormones (eg, parathyroid hormone [PTH], vitamin D, calcitonin) and (2) local regulation. Because all parts of the skeleton are not used equally, local mechanisms are required for appropriate local adaptation. For example, professional tennis players may have a 30% higher bone density in their dominant arm than in the nondominant one. Local mechanisms are thought to involve growth factors, which stimulate bone formation by increasing osteoblast proliferation and matrix biosynthetic activity.[1] This is not to say that systemic regulation of bone does not utilize growth factors. Indeed, there is evidence that the skeletal effects of at least some hormones (eg, growth hormone and PTH) are

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In This Issue

proteoglycan core protein. Overt chondrogenesis begins shortly after this condensation, with the synthesis and secretion of matrix rich in these macromolecules and others including types IX and XI collagen, proteoglycan link protein, and several less well characterized noncollagenous matrix proteins. Synthesis of the noncartilaginous proteins such as type I collagen ceases. This process produces the models, or so-called anlagen, of the future skeleton (Figure 1B).

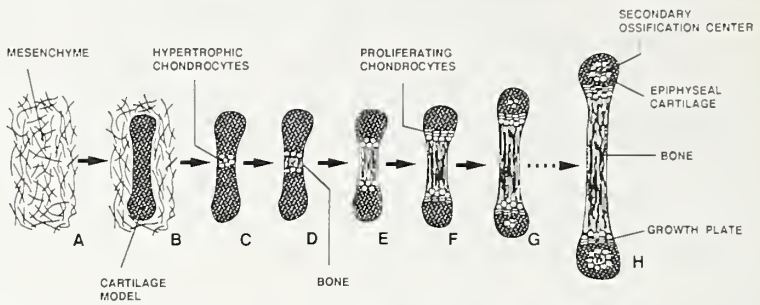

Figure 1 Bone formation and growth.

The Current Biologic Model

Soon after the cartilaginous models are formed, chondrocytes in the center of the anlagen begin to synthesize matrix molecules that are atypical of cartilage. These include type X collagen, fibronectin, and osteopontin. These changes signal the expression of a different type of chondrocyte, the hypertrophic chondrocyte (Figure 1C). More accurately, they indicate a switch in the phenotype expressed by the chondrocytes: Those synthesizing typical cartilage molecules are said to express the differentiated chondrocyte phenotype, whereas those synthesizing the atypical molecules express the hypertrophic chondrocyte phenotype. Other characteristics of the hypertrophic chondrocyte phenotype include a dramatic increase in cell size; expression of the activities of the enzymes alkaline phosphatase and carbonic anhydrase; and reduced synthesis of type II collagen and, possibly, of cartilage proteoglycans, proteoglycan link protein, and protease inhibitors that prevent vascular invasion. The net effect of these changes is that the matrix in the vicinity of the hypertrophic chondrocytes becomes susceptible to invasion by vascular cells penetrating from outside the cartilage model. As the cartilage matrix is degraded, the hypertrophic chondrocytes die and osteoblasts accompanying the vascular invasion begin to deposit bone matrix on fragments of incompletely degraded cartilage—ie, osteogenesis (Figure 1D). This produces hybrid trabecular structures containing a core of cartilage matrix and a surface of bone matrix. As the trabeculae are remodeled to complete the osteogenesis, the cartilage is degraded. Thus, chondrocyte hypertrophy initiates a cascade of events in which a space originally occupied by cartilage is completely replaced by bone.

As the center of the anlage is converted to bone, an ossification front is created between this newly formed bone and the remaining cartilage. The cartilage side of the front consists of hypertrophic chondrocytes preparing the matrix for vascular invasion, and the bone side is composed of osteoblasts depositing osteoids on spicules of incompletely degraded hypertrophic cartilage. This front spreads centripetally as progressively more of the chondrocytes hypertrophy and in turn more of the anlage is converted to bone (Figure 1D,1E). Most of the cartilage anlage and resident differentiated chondrocytes are consumed by this process; however, as this front nears the ends of a bone, a new element emerges. The chondrocytes distal to the front begin to proliferate and elaborate typical cartilage matrix before they hypertrophy (Figure 1F). This occurs directionally along the growth axis of the bone and pushes apart the cartilaginous ends of the bone, which are now known as epiphyseal cartilages (Figure 1F-1H). In other words, de novo chondrogenesis provides a new and continuous source of cartilage to be converted to bone as the ossification front progresses linearly. With time the de novo chondrogenesis (chondrocyte proliferation and matrix production) become incorporated into the ossification front as its leading edge, thereby creating an active growth plate. The bone thereafter grows because new cartilage is formed, hypertrophies, is degraded, and eventually is replaced by bone within the growth plate, which is only a few millimeters thick. The structure and functional correlates of the growth plate are depicted in Figure 2. The dynamic nature of the growth plate is illustrated in Figure 3.

Structural elements of the growth plate can be identified in humans as early as at 16 weeks' gestation. As long as de novo chondrogenesis continues, the growth plate remains active and

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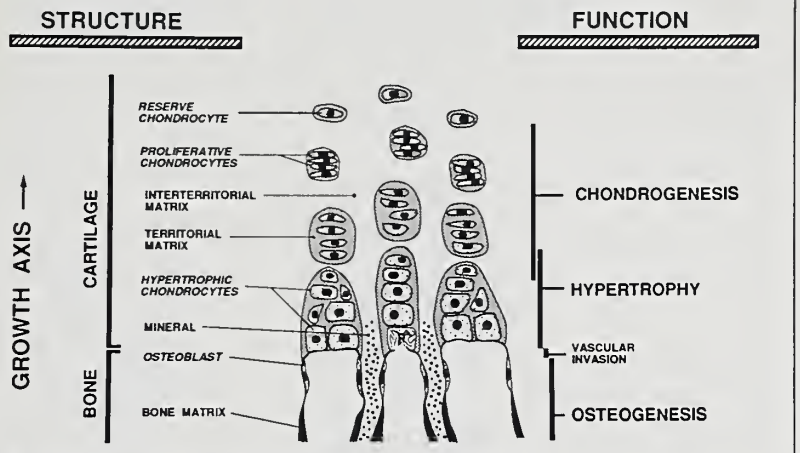

Figure 2 Growth plate structure-function relationships.

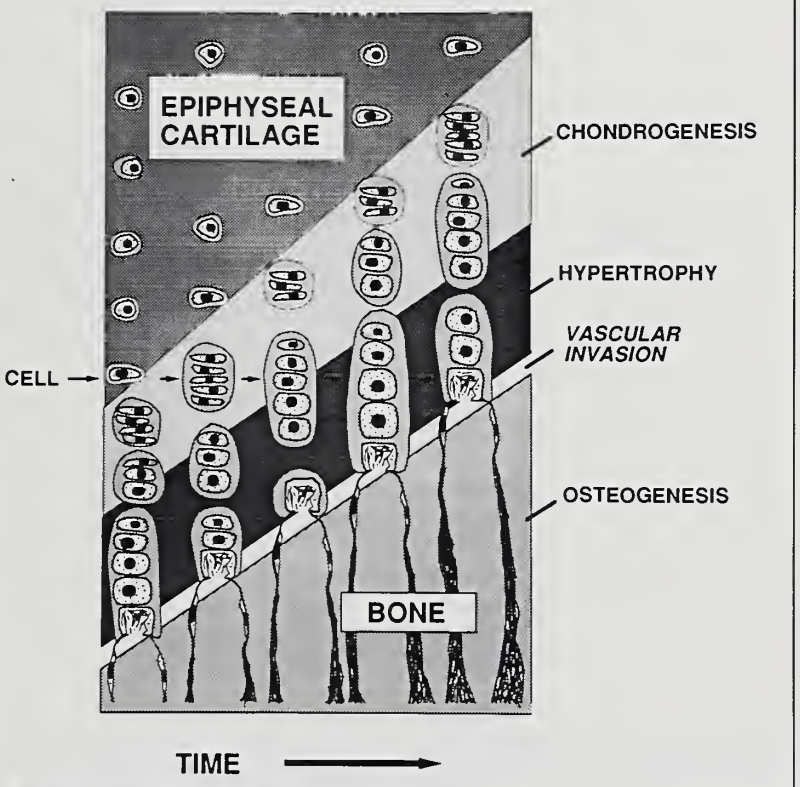

Figure 3 Growth plate dynamics.

the bone continues to grow. However, when it ceases, ie, at the end of puberty, the remaining epiphyseal cartilage undergoes hypertrophy and is replaced by bone.

The epiphyseal cartilages also give rise to secondary ossification centers during late fetal life and early childhood (Figure 1H). These develop in much the same way that the primary ossification centers arise in the center of the cartilage anlagen. They enlarge slowly as the cartilage is converted to bone and correspond to the "epiphyses" seen on X-ray.

Thus, the growth plate is a linear and dynamic structure in which de novo chondrogenesis provides a cartilage model that is modified through hypertrophy and eventually replaced by bone through osteogenesis. The coordinate regulation of these processes is not well understood, and a thorough discussion of the subject is beyond the scope of this brief review. Nevertheless, several important points can be made.

First, although osteogenesis alters the structural and mechanical properties of the skeleton, only chondrogenesis and hypertrophy contribute to physical lengthening. Second, these two processes reflect the progression of cells down a differentiation pathway (Figure 4). At the single cell level they correspond to the expression of the differentiated and the hypertrophic chondrocyte phenotypes, to which is added the element of proliferation. Thus, the factors that ultimately regulate bone growth are those that at the cellular level affect chondrocyte proliferation, expression of the two chondrocyte phenotypes, and progression of cells down the differentiation pathway.

Many factors have been shown to influence one or more aspects of this scheme through endocrine, paracrine, and possibly autocrine mechanisms. Indeed, at least three types of receptors have been either demonstrated or postulated to exist on chondrocytes. The first type includes cell-surface receptors that bind protein/peptide growth factors, such as growth hormone, insulin, insulin-like growth factor (IGF)-I, IGF-II, parathyroid hormone (PTH), epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor (TGF)-β. These receptors modulate signals across the plasma membrane that influence events within the cytoplasm and generate new intracellular signals. The second type of receptors, cytoplasmic receptors, respond to several steroid hormones (ie, glucocorticoids), estrogens, vitamin D metabolites, and retinoic acid derivatives. Activated hormone receptor complexes in the nucleus act by binding to specific DNA sequences and regulating gene transcription. The third type of receptor binds extracellularly to specific recogni-

tion sequences on matrix macromolecules, such as collagens, coupling them to intracellular cytoskeletal proteins. Some proteoglycans also function in this manner as do a large group of transmembrane molecules (integrins). These cell adhesion receptors permit the extracellular matrix, which is secreted and organized by the cells, to feed back to the cells and thereby affect their cytoskeletal organization.

Considerable interplay seems to occur among these receptor signalling mechanisms. For example, growth hormone is thought to modify the density of receptors for a number of peptide growth factors. Likewise, TGF-β may regulate the expression of integrin receptors. Furthermore, the signalling mechanisms are not distinct. EGF-like domains have been identified on several extracellular matrix molecules, and recognition sequences for integrin receptors have been demonstrated on one of the IGF-I binding proteins (BP-2).

The cellular regulation of chondrogenesis and chondrocyte hypertrophy is extremely complex. Although many hormones and growth factors are known to bring about various responses from chondrocytes, such as mitosis or synthesis of matrix molecules, the manner in which the many signals are integrated and related to each other and to the regulation of the overall developmental scheme is largely unknown.

This discussion provides an abbreviated and simplified view of how bones develop and grow. It also provides a framework in which to view the chondrodysplasias, inherited disorders of bone growth of which well over 100 distinct clinical entities are currently recognized. However, considering the complexity of the above scheme and the diversity of mutations that can occur within a single gene, the number of potential disorders, or more appropriately clinical phenotypes, is much larger. Candidate genes for mutations causing chondrodysplasias include those that are either expressed as components of one or more of the chondrocyte phenotypes or whose expression is involved in regulating chondrocyte proliferation or the progression of cells down the differentiation pathway. Much attention is currently focused on detecting mutations of the genes encoding cartilage matrix macromolecules, especially type II collagen. However, as the cellular and molecular biology of the overall process and its local regulation becomes better understood, the search will broaden, ultimately leading to results that will provide the basis for future approaches to therapy for short stature.

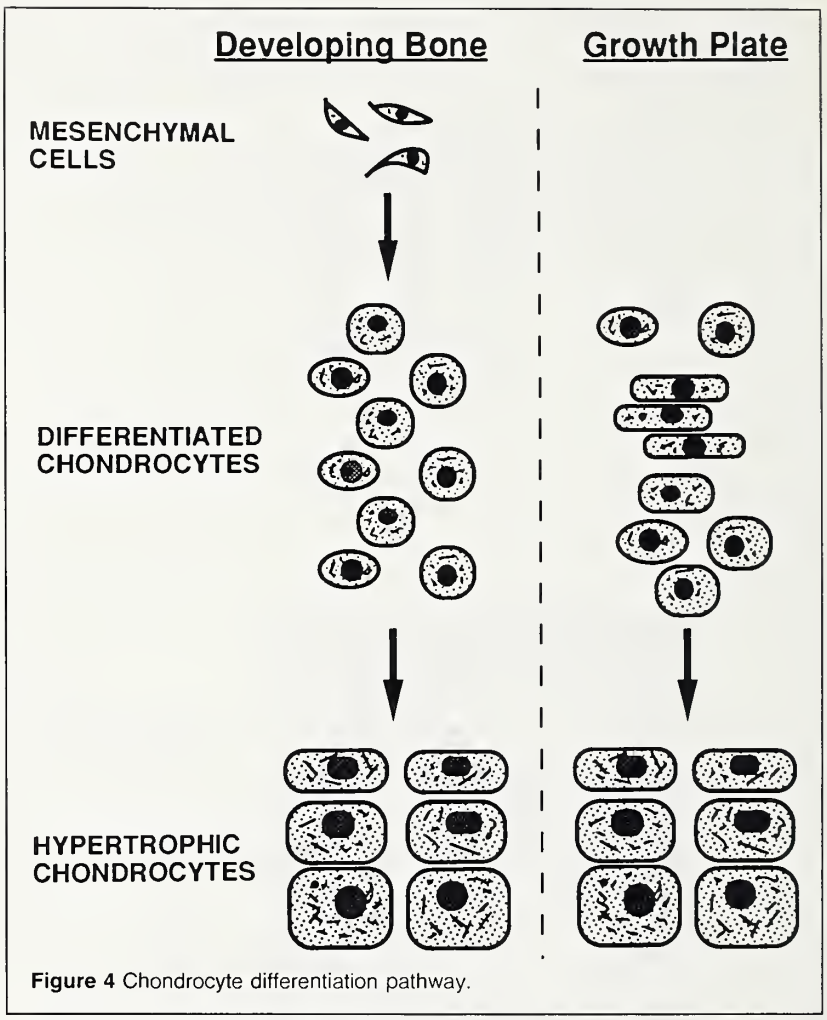

Figure 4 Chondrocyte differentiation pathway.

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Autocrine and Paracrine Aspects of Bone Metabolism

Continued from page 1

mediated by production of local growth factors under the influence of these systemic hormones. Recent findings—that skeletal tissue is a major storage site for growth factors and that bone cells in culture produce and respond to bone growth factors—support the concept that regulation of bone volume may depend on the local growth-promoting activities of bone-derived growth factors.

We will evaluate the potential role(s) of human bone-derived growth factors as determinants of local bone formation by discussing (1) growth factors stored in human bone; (2) growth factors produced by human bone cells; and (3) the biologic actions of human bone-derived growth factors.

Growth Factors Stored in Human Bone

Bone has the unique ability of self-regeneration in response to mechanical injury or tissue wasting. In recent years it has become apparent that these self-regenerative properties may result from the presence of bioactive polypeptide factors in the extracellular matrix of bone. Our studies during the past few years have centered on one such bioactive factor, skeletal growth factor (SGF).

SGF is present in high-molecular-weight forms in nondissociated extracts of both human bone and serum-free human bone cell-conditioned medium, apparently in complexes with binding proteins. Subsequently human SGF has been dissociated from these high-molecular-weight complexes and purified to homogeneity.[2] Structural studies of human SGF have revealed that the amino acid sequences of the amino terminal region and several tryptic fragments of human SGF were identical to the corresponding sequences of insulin-like growth factor II (IGF-II) from human serum, thus suggesting that SGF is very similar, if not identical, to IGF-II. During the purification of SGF from human bone matrix extract, we found evidence for the presence of additional growth factors. Characterization of these additional growth factor activities (Figure 1) revealed that:

1. Human bone matrix contains multiple growth factors, including IGF-I, IGF-II, transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF).
2. Human bone matrix does not contain detectable amounts of epidermal growth factor.
3. IGF-II and TGF-β1 are the two most abundant growth factors present in human bone matrix. IGF-I, PDGF, and bFGF are several-fold less abundant.[3]

The majority of TGF-β1 is present in an inactive (latent) form in human bone matrix extract under nondenaturing conditions. Latent TGF-β1, however, can be activated by treatment with acid, by certain proteases, or by deglycosylation. Several bioactive factors have also been identified in dissociative extracts of bovine and rat bones. These include bone morphogenic proteins, osteoinductive factor, osteogenin, and chemo-

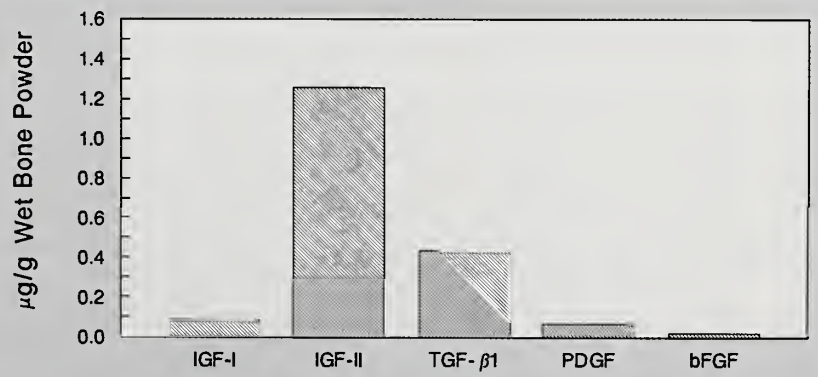

Figure 1 Relative distribution of growth factors in human bone. Matrix proteins were extracted from human bone powder by demineralization in a solution of 10% EDTA containing 4 *M* guanidine-HCl and protease inhibitors. The extracts were desalted and used for growth factor measurements using specific assays.

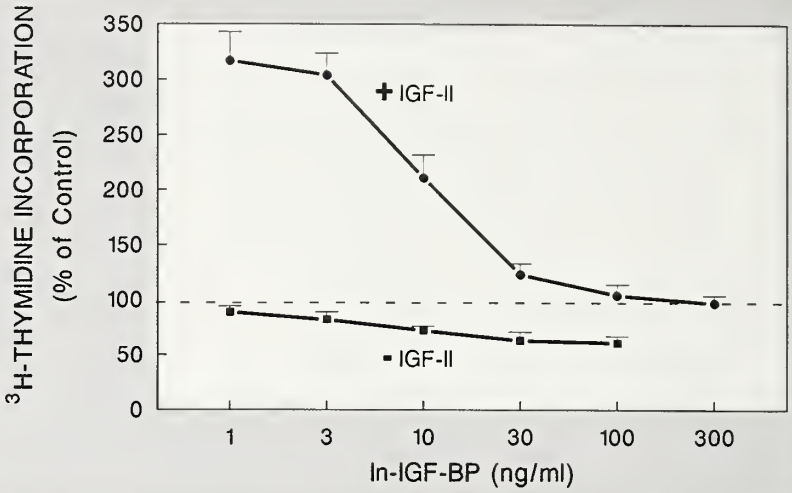

Figure 2 In-IGF-BP inhibits both basal and IGF-II-induced chick bone cell proliferation. The incorporation of [^{3}H]thymidine into DNA of chick calvarial cells was determined in the presence or absence (basal) of 3 ng/mL IGF-II and varying concentrations of In-IGF-BP. The values are means ± SD of six replicate wells. Basal and IGF-II-stimulated [^{3}H]thymidine incorporation was significantly inhibited ($P \leq 0.001$) by In-IGF-BP at 10, 30, 100, and 300 ng/mL.

tactic factors. Thus, skeletal tissues may constitute the single largest storage site for growth factors in the body.

Growth Factors Produced by Human Bone Cells

Human bone cells in culture produce a number of growth factors, many of which are known to be stored in human bone matrix, including IGF-I, IGF-II, TGF-β1, and PDGF. Bovine bone cells have been shown to produce bFGF, and since bFGF is found in human bone matrix, it seems likely that human bone cells also produce bFGF. Of these growth factors, IGF-II seems to be the most abundant mitogen produced by human bone cells. IGF-I is produced by human bone cells at 50- to 100-fold less concentration than IGF-II. As mentioned earlier, systemic hormones may modulate local bone formation at least in part through regulation of synthesis and release of bone growth factors. For example, PTH (which can also act as a bone cell mitogen) was shown to increase the release of IGF-I in rat bone cell cultures and the release of both IGF-I and IGF-II in mouse bone cultures.[4,5] Recent experiments have also shown that steroid hormone, 17β-estradiol increased the release of IGF-I, IGF-II, and TGF-β1 in the rat osteosarcoma cell line, UMR106.[6] These findings suggest that agents that stimulate bone formation may modulate their effects by altering the production of one or more bone growth factors in local skeletal sites.

We have recently shown that human bone cells produce, in addition to IGF-II, a binding protein known as inhibitory IGF-binding protein (In-IGF-BP). Studies on the *N*-terminal amino acid sequence and amino acid composition have revealed that In-IGF-BP purified from human bone cell-conditioned medium is unique, with limited sequence similarities to other known IGF-binding proteins.[7] Studies on the biologic actions of In-IGF-BP have revealed that it acts by inhibiting the binding of IGF-II to bone cell receptors. Figure 2 shows that 3 ng/mL of IGF-II stimulated bone cell proliferation (determined by [^{3}H]thymidine incorporation into DNA) 2.2-fold over controls in chick calvarial cells. The 3 ng/mL of IGF-II-stimulated bone cell proliferation was inhibited dose dependently with increasing concentrations of In-IGF-BP, and at 100 ng/mL, In-IGF-BP completely inhibited stimulation by 3 ng/mL IGF-II. In addition, In-IGF-BP also inhibited basal chick bone cell proliferation in a dose-dependent manner, with maximal inhibition of 40% at 100 ng/mL In-IGF-BP ($P<0.001$), thus emphasizing the importance of local (paracrine and autocrine) IGFs in bone cell proliferation. These findings are consistent with previous results showing that bone cells in culture produce IGF-I and IGF-II. Our recent findings have also shown that the production of In-IGF-BP by bone cells is regulated. For example, prostaglandin E_2 and dibutyryl cyclic AMP stimulated production in a dose-dependent manner.[7] These findings together suggest that In-IGF-BP may act as an important local regulator of IGF-II actions.

Biologic Actions of Human Bone-Derived Growth Factors

In recent years it has become evident that bone cells from nonhuman species may respond quite differently than human bone cells to growth factors. For example, TGF-β1 has been shown to be a potent mitogen for chick bone cells but has not been shown to stimulate human bone cell proliferation. Thus, for in vitro studies to be relevant to human physiology and pathology, human bone cells must be used. On the other hand, human bone cells are difficult to grow and thus there is a dearth of published reports on them. Work from our lab shows the effects of known bone-derived growth factors on proliferation of human bone cells isolated from trabecular bone of femoral head samples (Figure 3): 30 ng/mL of IGF-I or IGF-II doubled the stimulation of human bone cell proliferation, compared with control, with identical dose-response curves for IGF-I and IGF-II. The concentration required for half-maximal stimulation was estimated to be 25 ng/mL for either factor.[8] IGF-II also increased syn-

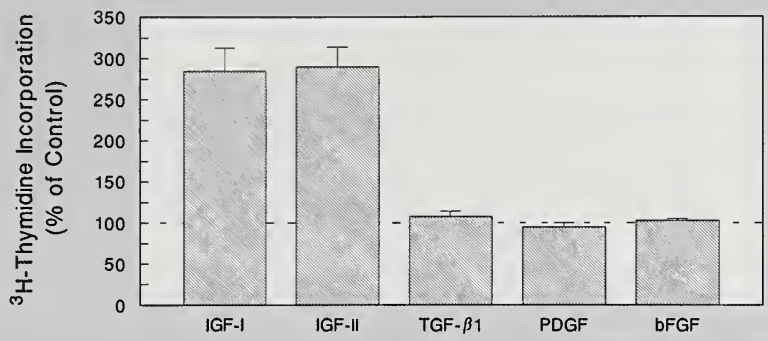

Figure 3 Effect of growth factors found in human bone on human bone cell proliferation. Values are means ± SEM of six replicate wells. Stimulation by 30 ng/mL IGF-I or IGF-II was significant at $P \leq 0.001$. TGF-β1, PDGF, or bFGF at 5 ng/mL had no effect on human bone cell proliferation under the culture conditions tested in this study.[8]

thesis of type I collagen and thus stimulated the differentiation of human bone cells. TGF-β1, PDGF, and bFGF had no effect on human bone cell proliferation under the conditions tested in this study. However, more recently, with a different set of culture conditions, we have found that PDGF and bFGF each stimulated human bone cell proliferation, whereas TGF-β1 did not stimulate human bone cell proliferation under any of the culture conditions we tested. TGF-β1 did stimulate production of type I collagen in human bone cells. Furthermore, in vivo stimulatory effects of TGF-β1 on bone formation have been reported by two different groups. Thus, each of the bone-derived growth factors identified in human bone matrix has been shown to increase proliferation and/or collagen synthesis in cells of osteoblastic lineage. We and others have proposed that these growth factors may act individually or in concert to stimulate the local bone formation.

We speculate that the bone-derived growth factors may act in an autocrine, paracrine, or delayed paracrine manner in the bone microenvironment. These growth factors are either incorporated into bone matrix or they diffuse to the extracellular fluid (Figure 4). On the other hand, growth factors secreted into extracellular fluid will have an acute autocrine or paracrine action on osteoblast-like cells. The finding that cells of the murine clonal osteoblastic cell line, MC3T3-E1 (representing relatively mature osteoblasts) both produce and respond to IGF-II

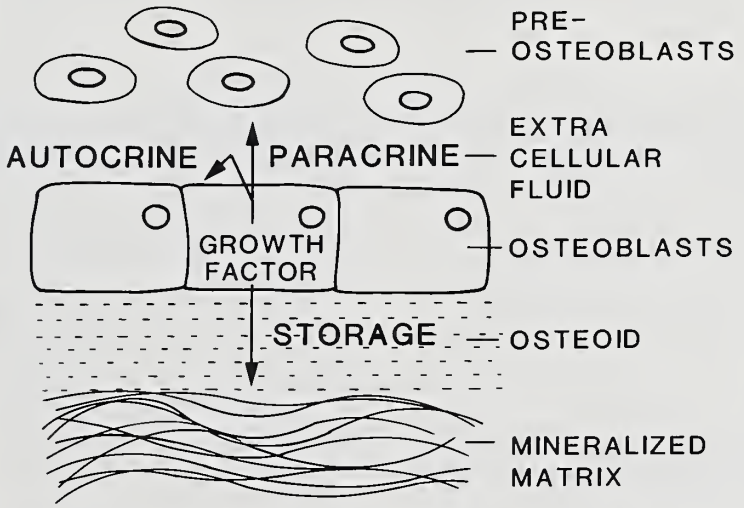

Figure 4 Model illustrating both storage of growth factors in bone and autocrine and paracrine actions of these factors on bone cells. Growth factors produced by osteoblasts can diffuse toward, and be deposited in, bone, or diffuse into the extracellular fluid to act on the same cell in an autocrine manner or on a nearby cell in a paracrine manner.

supports an autocrine action of IGF-II in bone cells. On the other hand, growth factors secreted into extracellular fluid could also act on nearby cells in a paracrine manner. For example, IGF-II produced by mature osteoblasts may act on nearby preosteoblasts as a paracrine agent to stimulate cell proliferation.

In contrast to these acute effects, growth factors stored in bone may also function as delayed paracrine agents, coupling bone formation to bone resorption.[9] Hence growth factors may be deposited for a time in bone and then released by osteoclastic bone resorption in a bioactive form to act on preosteoblasts and mature osteoblasts, thus allowing for site-specific replacement of bone that was lost to resorption (Figure 5).

Future Directions

In terms of what we know now and how this knowledge should be extended by future investigations, there is a considerable body of data on the regulation of serum IGF-I. In contrast, we know much less about the regulation of serum IGF-II or of the family of IGF binding proteins. The binding proteins probably function not only as carriers for the IGFs in serum but also as modulators of their actions. It would seem prudent to pursue the serum regulation of In-IGF-BP inasmuch as it can completely abolish the stimulatory effects of both IGFs. However, before embarking

on extensive studies of the serum IGFs and their binding proteins, methods must be developed that allow measurement of the IGFs specifically, without the potential artifacts of the binding proteins, and conversely, of the binding proteins without measuring the IGFs. It seems likely that in the past we have interpreted serum changes in IGFs when actually the changes were at least in part due to changes in the amounts of circulating binding proteins.

In past decades, efforts have concentrated on determining the regulation of hormones and hormone-like molecules in the circulation. More recently, the autocrine and paracrine actions of those hormones, which are actually local messenger molecules as well, have been studied. For example, vitamin D may have different effects when it behaves as a hormone and when it behaves as an autocrine or paracrine agent. The active metabolite to vitamin D, $1,25(OH)_2D_3$, is synthesized in the kidney and circulates as a hormone to regulate calcium metabolism and specifically to increase calcium absorption. However, $1,25(OH)_2D_3$ is not exclusively produced in the kidney. It is also produced in several other tissues, where it is thought to behave as an autocrine or a paracrine agent to facilitate the process of cell differentiation. Thus, as a hormone it regulates calcium metabolism at the level of both the gut and bone, whereas, as an autocrine and a paracrine agent, it may well act as a differentiation promoter.

Similarly, IGFs may also act as both hormones and local messengers. It is thought that the majority of the circulating IGFs are synthesized in the liver; however we now know that many other organs produce the IGFs, perhaps for use as autocrine and paracrine messenger molecules. Regulation of liver production of IGFs for hormonal use may be quite different from the autocrine/paracrine regulation of the IGFs. Thus we cannot understand the IGFs by looking only at serum; we must also examine the local effectors of IGF secretion in various tissues. It is possible that the regulation of IGFs at all local levels, including the gene level, may be different in the liver than in other tissues. Such studies of the paracrine/autocrine actions of the IGFs will be technically difficult, because for true clinical relevance, they must be studied in vivo; unfortunately, technology now permits their study only in vitro.

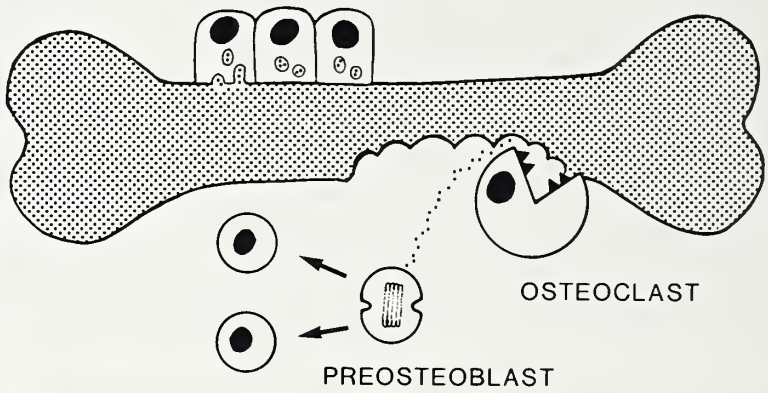

Figure 5 Model illustrating the delayed paracrine actions of growth factors stored in bone. Growth factors produced by osteoblasts are deposited in bone for later release by osteoclastic resorption to act on preosteoblasts and mature osteoblasts, thereby affecting site-specific bone replacement.
(Reprinted with permission from Farley JR et al and Grune and Stratton Inc.[9])

Thus far we have discussed general opportunities for future investigations of the IGFs and their corresponding binding proteins. More specifically, we would suggest that some emphasis should be placed on examining the role of the IGFs in mediating the tissue-promoting (anabolic) activities of physical exercise. It is now well established that exercise promotes large changes in growth hormones. It is also possible that locally, exercise somehow signals an increase in IGF production or action. If so, it could well be that exercise increases tissue anabolism, not only by the hormonal actions of circulating IGFs, but also by the actions of locally produced IGFs in response to local mechanical loads.

Another important area in which growth factors may be operative is in the determination of peak bone mass, which occurs at about 25 to 30 years of age. The higher the peak bone mass, the less likely a patient will, during aging, lose bone down to a level where fractures begin to occur. Thus, one way to mitigate osteoporosis would be to promote a high peak bone mass. It seems likely that growth factors, and perhaps the IGFs, are involved in determining peak bone mass. These possibilities could be explored by observing restriction fragment length polymorphism (a clinical study). Another approach would be to correlate bone density to either serum or bone IGFs; individuals with a high peak bone mass would be expected to have high IGF levels in either serum or bone.

IGFs may also play a role in coupling bone formation to resorption. The hypothesis is that whenever there is an increase in bone resorption, there will, after a brief delay, be a corresponding increase in bone formation to maintain a constant appropriate bone mass. On the other hand, during estrogen deficiency after menopause, there is a large increase in bone resorption with an inadequate increase in bone formation, causing a progressive loss of bone. In vitro studies in rats suggest that bone cells make IGFs as well as TGF-β1 in response to estrogen, leading to the hypothesis that during estrogen deficiency, growth factors drop to lev-

els insufficient to mediate a coupled increase in bone formation. The importance of estrogen deficiency in the development of osteoporosis cannot be overemphasized, and any clarification of the role of estrogens on growth factor and binding protein production by bone cells would be an important advance.

Similarly, we know that androgen deficiency causes a marked bone loss in males and that androgens are potent mitogens for bone cells. However, the mechanism of growth factor involvement in the androgen response is largely unknown. These androgenic actions are not only relevant to the aging male in whom androgen deficiency probably contributes to bone loss, but also to adolescent children during the growth spurt. In the same manner, estrogen deficiency accounts for menopausal bone loss as well as decreased adolescent growth spurt.

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GnRHa Therapy: Questions and Answers

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Editor's note:
There is considerable interest today in the use of GnRH analogs for the treatment of sexual precocity. There is also much confusion about how to monitor for suppression of LH and FSH when the various analogs are used. In an attempt to clarify this confusion, Dr. Paul Boepple succinctly answered several questions that I posed to him. Readers may benefit from these responses, with which I agree. Dr. Boepple previously published an article titled, "Sexual Precocity, GnRH Analogs, and Growth" in this journal (Vol. 5, No. 1, March 1989).

Robert M. Blizzard, M.D.

How do the potency and pharmacokinetics of different gonadotropin-releasing hormone (GnRH) analogs correspond with clinical observations?

To date only GnRH agonist (GnRHa) analogs are used in clinical situations. These agents stimulate the release of leutinizing hormone (LH) and follicle-stimulating hormone (FSH) and induce gonadotropin desensitization with continuous exposure of high concentrations to the pituitary. Desensitization thus depends on the potency of the LHRHa, the frequency of administration, bioavailability (eg, only a small fraction of an intranasal dose is absorbed), and clearance. Less potent and more rapidly cleared agonists are less effective in inducing complete suppression. *Importantly*, some agonists given intranasally or subcutaneously are cleared so rapidly that the pituitary is not completely suppressed and responds to the next agonist dose with increased release of LH and FSH.

What methods of monitoring do you recommend?

"Undetectable" levels of estradiol in serum are insufficient proof of suppression since most radioimmunoassays are not sensitive enough to measure small but clinically significant levels. The best evidence for suppression is failure of the LH to rise when the pituitary is "challenged," either with LHRH or LHRHa, intravenously. FSH measurement is desirable but not absolutely necessary. The physician must ensure that the LH assay used *does not* measure α-subunits, as α-subunits continue to be released even when LH and FSH are suppressed, and LH assays that have cross-reactivity between LH and α-subunits can be misinterpreted.

What about comparisons of the dose-effectiveness of the various analogs?

Relative potencies have been determined primarily in in vitro assays. While these cannot be translated directly for use in humans, the doses required clinically follow the rank order of these potency determinations. For each LHRHa the dosage must be established by monitoring for complete suppression in each patient.

Must suppression be complete or is it acceptable for small amounts of estrogen or testosterone to be secreted?

Opinions vary; however, our group believes that complete suppression is exceedingly important. The failure of medroxyprogesterone acetate or cyproterone acetate to have a significant impact on final height in precocity may very well be a result of their inability to achieve complete suppression of gonadal activity.

Is the timing for biochemical evaluation of complete LH suppression in relation to the time of agonist administration important?

Yes. Randomly measured gonadotropin and sex steroid levels may be low, even in incompletely suppressed patients. LH, FSH, and sex steroids may rise for several hours after the daily dose of LHRHa and then return to "suppressed" levels before the next dose. However, since the time course of the pituitary and gonadal responses are different, apparent discrepancies may arise (eg, in a single random sample, estradiol or testosterone may be increased after LH and FSH have returned to baseline). Monitoring must be done after a "pituitary challenge" to prove that the axis is suppressed.

Is there a difference in the way children of various height ages or bone ages (BAs) respond to the analog?

LHRHa can produce complete suppression of LH secretion regardless of age, if an adequate dosage is used. However, children with BA $\leq$12 years will grow more rapidly with treatment than children with BA >12 years. It stands to reason that the growth patterns will be different. Without sex steroids younger children have a decrease in growth velocity but grow at age-appropriate rates, and their BA maturation slows to a normal rate but does not stop. Growth is slower in children with BAs in the late pubertal range, but their BAs show very little progression when sex steroids are removed. Even though growth velocity is <4 cm/yr in these patients, epiphyseal fusion is delayed and final heights surpass pretherapy predictions.

Is LHRHa indicated in growth hormone (GH)-deficient patients who have entered adolescent development and who are short?

Theoretically this might be of benefit. However, I cannot advocate this now outside an investigational protocol. The data to support its use are not available. Similarly, its use in other causes of short stature, with or without GH, cannot be currently approved. Appropriate protocols to answer these questions are needed.

In Future Issues

IGF-Binding Proteins: Their Physiological and Clinical Importance
by Michael Ranke, M.D.

Genomic Imprinting
by Judith G. Hall, M.D.

Complications of Excessive GH in Acromegaly
by Mark Hartman, M.D.

Robinow Syndrome: An Update
by Meinhard Robinow, M.D.

Childhood Obesity
by William Dietz, M.D.

Special Report

American Society of Human Genetics Meeting

November 12-15, 1989, Baltimore, Maryland

Judith G. Hall, M.D.
Associate Editor
Growth, Genetics, and Hormones

Among many outstanding symposiums and presentations, some highlights of this meeting included a report by Tsui of the isolation of the cystic fibrosis gene on chromosome 7. According to Tsui, 70% of individuals carrying the cystic fibrosis gene have the same defect (allele). Recent work has attempted to characterize the other 30%. With the isolation of the gene, work on its function and on the pathogenesis of cystic fibrosis becomes the central issue, along with the question of whether newborn screening should be adopted.

Nicholls and co-workers reported several patients with Prader-Willi syndrome who, instead of having deletions of chromosome 15, had inherited two copies of chromosome 15 from their mothers. Both isodisomy and heterodisomy of maternal chromosome 15 were reported. This suggests strongly that it is the *absence* of the specific locus in the p11-p13 region on chromosome 15 that is responsible for producing the syndrome.

Verlinsky and co-workers presented a new approach to preconception prenatal diagnosis, using in vitro fertilization techniques prior to fertilization. After removing the first polar body, they were able to analyze its DNA using the polymerase chain reaction, to see whether it carried an abnormal allele. They were looking for the abnormal allele of the α-1 antitrypsin gene, but almost any other characterized gene could be analyzed in the same way. If the abnormal allele is in the polar body, the egg will be left with the normal gene; thus prenatal diagnosis can be accomplished prior to fertilization and implantation. The problem with the technique is that (1) crossover occurs with meiosis, and (2) the polar body may be heterozygous.

A large symposium was held on the status of the human genome project. Both the NIH and the Department of Energy are advocating an improvement of techniques for mapping and isolating genes, particularly with regard to technology, management of large amounts of information, and communication between researchers. In addition, a number of issues have arisen relating to the ethics of the research itself and to the ethical uses of the information that is obtained.

Effects of Chronic Overproduction of GH and IGF-I in Transgenic Mice

An animal model of gigantism was created a few years ago by developing a transgenic mouse that expressed high levels of growth hormone (GH). The animals exhibited a dramatic increase in size and weight as well as a variety of complications (*Nature* 1982;300: 611-615). Because nonmurine GH genes were used and also because the GH was expressed in many organs, it was not known if the pathologic effects were due to chronically high levels of circulating GH or to other factors. To resolve this question, another transgenic mouse model was created in which hypothalamic growth hormone releasing factor (GRF) was overproduced. This caused hyperplasia and hypertrophy of pituitary somatotrophs with secretion of excessive amounts of endogenous GH in the transgenic mice (*Nature* 1985;315:413-416). Since many of the effects of GH are mediated by insulin-like growth factor I (IGF-I), Quaife et al produced another transgenic mouse in which IGF-I is overproduced. They also compared a number of parameters in the three transgenic mouse models and controls.

In general, animals with high levels of GH exhibited similar features regardless of the source of the "trans" GH gene (rat, human, bovine), the promoter that regulated its expression (metallothionein or albumin promoter), or whether the GH excess was endogenous from GRF overstimulation or from expression of a foreign GH gene. When these animals (high-GH animals) were compared to animals with high IGF-I levels that resulted from IGF-I transgene expression (high IGF-I animals), several differences were detected. Although both animals weighed much more than controls, linear skeletal growth was increased in the high-GH animals but not in the high-IGF-I animals. In addition to GH, insulin levels were greatly increased in the high-GH animals, whereas both were subsequently reduced in the high-IGF-I animals. Hepatic and renal pathologic lesions were seen the high-GH animals but not in the high-IGF-I animals. The lesions consisted of hyperplasia, hypertrophy, and sclerosis in the liver and increased glomerular size, mesangial hypercellularity, and glomerular sclerosis in the kidneys. The renal lesions resembled those found in diabetes. Thickening of the skin due to an increase in dermal and subdermal fat was observed only in the high-IGF-I animals. Cholesterol tended to be elevated in the high-GH animals, whereas triglycerides were elevated in the high-IGF-I animals. Finally, survival was reduced in the high-GH animals; 60% were alive at 6 months of age compared with 100% of controls. The deaths were attributed to renal disease. Survival was not examined in the high-IGF-I animals.

The authors concluded that chronically elevated GH has detrimental effects on a number of organ systems and that many of these effects are not mediated by IGF-I alone. They acknowledged many differences between transgenic models of GH elevation and the clinical administration of GH in humans but cautioned that the long term effects of GH treatment in children must be carefully evaluated.

Quaife CJ, Mathews LS, Pinkert CA, et al. *Endocrinology* 1989; 124:40-48.

Editor's comment—*As the authors point out, the experimental models employed in this study of the effects of chronic GH and IGF-I stimulation differ both qualitatively and quantitatively from the clinical setting in which GH is administered to children. Nevertheless, as temptation grows to use higher and more frequent doses of GH to treat short stature, especially short stature not due to GH deficiency, the caution urged by the authors should be remembered.*

*One of the more interesting observations from the study is that even though IGF-I levels were increased 1.5-fold and body weight 1.4-fold over controls in the mice expressing the IGF-I transgene, linear skeletal growth was not increased. These results differ from those reported by Guler et al (*Proc Natl Acad Sci USA *1988;85: 4889-4893), who infused GH or IGF-I into hypophysectomized rats and found increased linear bone growth in both cases. Because of differences in design, the results of the two studies cannot be directly compared, but both sharpen the debate over how GH acts to promote linear skeletal growth.*

William A. Horton, M.D.

Do Extracellular Matrix Proteins Exhibit Growth Factor Activity?

Historically, growth factors were identified as circulating proteins and peptides that influenced cell division and differentiation. It was later determined that many growth factors are generated and act locally, ie, paracrine and autocrine growth factors. There is now growing evidence that many extracellular matrix proteins contain functional domains with growth factor activity.

Engel recently reviewed the situation with regard to epidermal growth factor (EGF) domains in several large matrix proteins. EGF is a small peptide (53 amino acid residues) that is known to promote mitosis in many cell types through interaction with a specific cell

Do Extracellular Matrix Proteins Exhibit Growth Factor Activity? continued from page 11

membrane receptor. Three large multidomain extracellular matrix proteins contain EGF-like domains. They are typically repeated manyfold in accessible regions of the molecules. The first protein is laminin, which is found in basement membranes; the second is tenascin, a widely distributed matrix protein; and the third is thromboplastin, found in platelets and vascular walls. When these proteins are used as culture substrates, cells generally grow even in the absence of other growth factors, eg, those supplied by serum.

Engel suggests that these proteins may stimulate growth early in development, before the EGF-like domains are covered up by other components of extracellular matrix, and especially during tissue repair, when they are exposed by the tissue damage. In this fashion they are able to provide highly specific and very localized signals for growth and repair that cannot be achieved by diffusible growth factors.

Engel J. *FEBS Lett* 1989;251:1-7.

Editor's comment—*This article brings attention to three relatively new concepts. The first is that large proteins often exhibit modular construction with different modules having different functions, ie, cell adhesion, molecular interaction, structural integrity, growth promotion, etc. Second, the same module may be shared by different molecules. Third, the extracellular matrix does more than occupy space between cells. Overall, this article contributes to a more complete picture of how the growth and differentiation of individual cells are regulated.*

William A. Horton, M.D.

The Half-Life of Exogenous GH After Suppression of Endogenous GH Secretion with Somatostatin

Suppression of endogenous growth hormone (GH) secretion by an infusion of somatostatin (SRIF, IV, 50 $\mu g/m^2$/hour) permitted measurement of the half-life of exogenously administered GH. Fourteen studies were performed in six male subjects (five normal adult males, one adolescent with GH deficiency following cranial irradiation). One hour after the start of the SRIF infusion, a bolus of monomeric biosynthetic GH (Nordisk, Gentofte, Denmark) was injected intravenously at a dose of either 500 mU (n=9) or 50 mU (n=5). Serum GH was measured over three consecutive 30-minute periods at intervals of 1, 5, and 10 minutes, respectively. Both immunoradiometric assay (IRMA) and enzyme-linked immunosorbent assay (ELISA) were used for the GH measurements. The half-life of GH was calculated from the logarithm of serum GH concentrations during the 90 minutes. A control study with GH, 500 mU, after 1 hour of saline infusion was performed twice in three subjects.

The serum GH was undetectable at the end of the first hour of SRIF. The distribution phase of injected GH was complete by 6 minutes. The mean half-life of GH was 9.3 ± 1.45 min after 500 mU and 8.5 ± 1.5 min after 50 mU. Combining the data from both studies gave a mean half-life of 8.9 ± 1.5 min. Replacing SRIF with saline did not change the results.

Hindmarsch PC, Matthews DR, Brain CE, et al. *Clin Endocrinol* 1989;30:443-450.

Editor's comment—*There have been many discrepant studies suggesting that the half-life of circulating GH was more than 15 minutes. These previous studies measured the decay of either a small bolus of radiolabelled GH or a very large bolus of unlabelled GH in subjects whose endogenous secretion of GH had not been suppressed. The technical conditions of the present study—no interference from endogenous secretion; use of monomeric hGH at physiologic doses; serum GH measured by two sensitive and reliable methods—are clearly more appropriate.*

Knowing that the half-life of the circulating GH is around 8 to 9 minutes is of clinical importance. It suggests that a 10-minute sampling interval may be necessary to properly evaluate the profile of endogenous GH secretion and that the usual 20-minute interval of sampling may be insufficient.

Jean-Claude Job, M.D.

Second editor's comment—*The authors note that the data reported in the above abstract are at variance with other reports. Using variable techniques, the half-lives of circulating GH have been found to be between 7 to 51 minutes; at least five previous articles reported that the half-life is greater than 15 minutes. The authors attribute the difference in their results to the use of SRIF. However, in an article by Faria et al (*J Clin Endocrinol Metab *1989;68:535) in which SRIF and endogenous secretion of GH under GH releasing hormone stimulation were studied, the in vivo half-life was found to be 18.9 ± 0.8 min by monoexponential analysis, and 3.5 ± 0.78 min and 20.7 ± 0.7 min by biexponential curve fitting. Both studies tested normal young adults except for one patient in Hindmarsch's study who was GH deficient. The reason for differences in the results in these studies is unclear. The reader needs to be aware that a consensus has not been reached regarding the half-life of circulating GH.*

Robert M. Blizzard, M.D.

Partial GH Deficiency in Short Prepubertal Children with Intra-uterine Growth Retardation

Three European groups of pediatric endocrinologists have recently emphasized the frequency with which a low or abnormal secretion of growth hormone (GH) is found in children with intrauterine growth retardation (IUGR), with or without Silver-Russell syndrome (SR).

Albertsson-Wikland reports data on 16 IUGR children with lengths 3 SD below normal at birth. These children were studied between 2 to 6 years of age, when their heights were 2.7 to 5.5 SD below normal. (In addition, 6 children had features of SR.) Their mean GH response to an arginine-insulin test was 15.7 ± 7.2 ng/mL; five of these had peak responses below 10 ng/mL. A 24-hour GH profile (withdrawals every 30 min) in 3 of the 6 SR patients and in 2 of the 10 other IUGR children showed low spontaneous secretion. Most of the other children showed minor disturbances in their circadian rhythm of GH secretion. All were treated with GH, 0.1 IU/kg/day, resulting in an average increase in growth velocity of 3.7 cm and 3.0 cm in the SR and the other IUGR children, respectively, during the first year of treatment. The gain in height was negatively correlated with the 24-hour GH secretion, evaluated by the area under curve (r = -0.56, $P<0.05$), but not with the peak result of the arginine-insulin stimulation test.

Rochiccioli et al studied 24 prepubertal IUGR children born with lengths below the 10th percentile for gestational age. At the time of the study their mean age was 5.5 years and their mean height -3.3 SD. One or two GH stimulation tests (glucagon-betaxolol, clonidine-betaxolol, or arginine-insulin) and a 24-hour (20-30 min sampling) profile of serum GH were performed in each patient. Of the 24, 7 had both a 24-hour integrated concentration of GH below 1.5 ng/mL and GH peaks not exceeding 5 ng/mL at the two stimulation tests. Another 9 had low integrated circadian concentrations, with either normal (n = 4) or low (n = 5) peak responses to stimulation. Only 8 had both normal responses to the stimulation tests and normal spontaneous GH secretory profiles. Of the 24, 9 (unclear which children) were treated with GH, 0.4 IU/kg/week, and had an increase in growth velocity from 3.5 ± 0.8 cm/year before GH to 7.0 ± 0.9 cm/year during the first year of treatment.

Stanhope and associates report data on 31 IUGR prepubertal children with mean age 6.0 years, mean height -2.84 SD, mean birth weight -2.82 SD, and mean growth velocity -0.76 SD, during the year preceding the study. Seventeen had signs of SR. GH secretion (15 min sampling) was determined overnight (8 P.M. to 8 A.M.): 4 of 31 had no spontaneous GH peak above 10 ng/mL and thus were considered to be GH deficient. Nine (8 with SR) had a single nocturnal pulse of GH. A therapeutic trial of GH was performed in 23 patients, with randomization to two clinically similar groups receiving either 15 IU/m^2/week or 30 IU/m^2/week of GH, by daily SC injections (approximately 0.45 and 0.90 IU/kg/week). Short-term mean results were: in the low-dose group, an increase of height velocity from -0.61 to +1.09 SD for 0.82 year; in the high-dose group, an increase from -0.61 to +3.48 SD for 0.92 year. The authors conclude 1) that GH deficiency—mainly abnormal rhythm of nocturnal GH secretion—is apparently common in growth retarded children with IUGR; 2) that the short-term effect of GH in these patients is positive and dose dependent; 3) that these initial results cannot determine whether GH treatment may improve the final height of IUGR patients, some of whom may have an accelerated skeletal maturation and an early onset of puberty.

Albertsson-Wikland K. *Acta Paediatr Scand (Suppl)* 1989; 349:35-41.

Rochiccioli P, Tauber M, Moisan V, Pienkowski C. *Acta Paediatr Scand (Suppl)* 1989;349:42-46.

Stanhope R, Ackland F, Hamill G, et al. *Acta Paediatr Scand (Suppl)* 1989;349:47-52.

Editor's comment—*Although these three studies differ in terms of protocol, their results are similar. They clearly show that some degree of abnormality in the secretion of GH is found, more often than previously reported, in very short children born small-for-date, irrespective of whether they have the features of Silver-Russell syndrome. In these children frequent circadian or nocturnal measurement of the serum GH levels is perhaps a better way to evaluate GH secretion than the usual stimulation tests. However, nothing is known at present about the long-term usefulness of GH therapy in non-GH-deficient IUGR children. A dose dependency may exist during the first year of treatment, but beyond this time data do not exist. We can conclude that studies such as these in IUGR children are extremely useful, but that they must be developed and conducted in long-term, controlled protocols. We cannot at present extend the data from these trials to conclude that the use of GH in endocrinologically normal children with short stature of prenatal onset is efficacious.*

Jean-Claude Job, M.D.

Homeotic Gene Expression in Vertebrates

A fundamental question in developmental biology concerns how a cell knows where it is, relative to other cells and to the overall body plan. Position signalling (as it is called) is very important during early embryologic development and also in linearly ordered processes such as skeletal growth. Much is known about position signalling in lower species, such as *Drosophila*, in which so-called homeotic selection genes appear to serve as master genes controlling expression of many other genes in the developing embryo. These master genes contain highly conserved (homeobox) sequences that code for DNA binding protein domains and are organized as clusters of contiguous genes on chromosomes. Their expression is segmentally distributed, providing information about the anterior-posterior position of cells within an embryo. Interestingly, there is a spatial relationship between the chromosomal order of the genes and the location of their expression in the embryo, such that the genes within a homeobox are sequentially expressed congruently with their anterior to posterior expression in the body.

Homeotic genes and gene clusters have been identified in higher species, including humans, but their functional similarity has been questioned because genesis of vertebrate and insect bodies differs so much. In particular, segmentation, which demarcates the limits of expression of the insect homeotic genes, has been thought to occur in a different fashion in vertebrates. However, a report by Wilkinson et al suggests that segmental expression of homeotic genes does occur in vertebrate embryos. Using in situ hybridization, these investigators demonstrated that expression of four contiguous murine homeotic genes (Hox 2.1, 2.6, 2.7, 2.8) exhibited a segmental distribution in the hindbrain of 9.5-day-old mouse embryos. The anterior limits of expression jumped by two segment intervals and the order of segmental expression corresponded to the chromosomal order of the gene loci. Hence, as with *Drosophila*, there is a physical relationship between the chromosomal order of gene loci and their segmental expression along the anterior-posterior axis of the early embryo.

Wilkinson DG, Bhatt S, Cook M, et al. *Nature* 1989;341:404-409.

Editor's comment—*The segmental expression of homeotic genes in the mouse hindbrain seems far removed from growth in humans. However, as one strives to understand human growth and development at the cellular and molecular levels, one becomes more dependent on knowledge acquired from lower organisms. Finding similarities between man and distant species in fundamental processes, such as positional signalling, greatly facilitates this task.*

William A. Horton, M.D.

Strategies for Optimizing Growth in Children with Kidney Transplants

In an attempt to diminish the growth failure that occurs post-organ transplantation in children with graft acceptance, but who are on low-dose steroids, the authors attempted to use cyclosporine as the primary immunosuppressant. Of 53 patients, 23 were able to discontinue prednisone and be maintained on cyclosporine monotherapy. Of these, 9 had to return to prednisone after a mean of 9 months (3-24 months). The other 14 remained off prednisone without an episode of rejection.

L-DOPA stimulation was used to evaluate growth hormone (GH) release. All patients were receiving >5 mg of prednisone daily; four patients had peak values <10 ng/mL GH. Standard deviations for height were evaluated in 15 patients who were off prednisone for at least 6 months; the SD scores improved in all.

Four pubescent children with growth retardation, requiring prednisone, received recombinant human GH (rhGH) in an attempt to stimulate growth. Three of these were believed to have accelerated growth.

The authors concluded that cyclosporine can produce long-term graft survival when used alone in some patients. Catch-up growth occurs in patients able to discontinue prednisone, and the potential of rhGH to improve post-transplantation growth in children needs further exploration.

Tejani A, Butt KMN, Rajpoot D, et al. *Transplantation* 1989;47:229.

Editor's comment—*The observations of Tejani et al conform with data collected through the years concerning catch-up growth that occurs when glucocorticoids are discontinued. The surprising observation is the increase in growth that occurred in three of the four patients on low-dose steroids who received rhGH. The effect of GH administration to patients with chronic renal disease and its growth promoting effect has previously been reported by Koch (*Pediatr Res *1988;23:541A).*

The data in this report are preliminary, and more such studies are needed to clarify the usefulness of rhGH therapy in patients with chronic renal disease. Significant new data will be forthcoming within the next year or two. In the meantime every effort should be made to minimize the amount of steroid used in such patients.

Robert M. Blizzard, M.D.

Effects of Different Oestrogen Doses on Final Height Reduction in Girls with Constitutional Tall Stature

Gruters et al have studied the effects of two different dosages of ethinylestradiol (EE) on final height reduction in German girls whose final height prediction exceeded 3 SD (>180 cm) above the mean. Group 1 (38 girls) at the University Children's Hospital in Gottingen received a daily dose of 0.3 to 0.5 mg EE, while 44 girls (group 2) at the University Children's Hospital in West Berlin received 0.1 mg EE daily. Both treatment protocols utilized daily estrogen administration in conjunction with 10 mg medroxyprogesterone acetate for 5 to 7 days every 4 weeks to induce cyclic bleeding. Subjects were examined every 3 months and bone age (BA) was determined by the Greulich and Pyle method every 6 months.

Treatment was discontinued at BA-15 years in group 1 and after two successive BA determinations ≥15 years in group 2. Final height was measured in all girls ≥18 years (mean, 20.2 years). Standing height was measured with a calibrated stadiometer and predicted height was estimated according to Bayley and Pinneau tables.

At the onset of treatment there were no differences in chronologic age, BA, height, growth velocity, or height prediction between the two groups. Growth velocity was significantly reduced by estrogen in both groups.

Although duration of treatment was longer in group 2, the cumulative estrogen dose was lower in group 2 than in group 1. From the predicted final height the mean reduction was 4.9 ± 2.6 cm in group 1 and 5.1 ± 2.4 cm in group 2. Final height was reduced more in each group when the treatment was started at BA <13 years. No side effects were observed in either group.

Gruters A, Heiderman P, Schludter H, Stubbe P, Webber B, Helge H: *Eur J Ped* 1989;149:11-13.

Editor's comment—*This article reports that in a large sample of tall girls, different doses of EE had similar effects on final height reduction. Thus, as the authors point out, it would seem prudent to utilize the lowest possible dose of estrogen in an attempt to minimize possible side effects—such as thromboembolism, hypertension, and increased body weight—that are known to be dose dependent. A prospective randomized clinical trial to determine the lowest effective dose is needed.*

William L. Clarke, M.D.

Effects of Oestrogen Treatment on the Proportionality of Growth in Tall Girls

Hermanussen et al studied the effects of estrogen therapy (conjugated estrogen, 7.5 mg/d, plus cyclic gestagens) on standing height and lower leg length in 17 girls with tall stature and compared those results to a control group of 17 healthy untreated tall girls. The heights of all girls exceeded 2 SD for age, or their predicted adult height exceeded 182 cm. All were measured weekly or monthly using a Harpenden stadiometer. Knemometry, a noninvasive technique, was used to measure the lower leg length in the sitting position. Growth rates were calculated using linear regression analysis.

Estrogen treatment led to a significant reduction of lower leg length increment in the treated girls. Standing height velocity dropped from 150 to 122 μm/d in the estrogen-treated girls. The decrease in standing height velocity was explained by a marked inhibition of lower leg growth velocity, from 42 to 30 μm/d. No differences in trunk growth velocity were detectable. According to the authors, these findings suggest that pharmacologic doses of estrogen act locally at the level of epiphyseal growth and, therefore, girls who have passed mid-puberty—when most peripheral growth has been completed—would not be expected to benefit significantly from high estrogen treatment.

Hermanussen M, Geiger-Benoit K, Burmeister J. *Euro J Ped* 1989;149;14-17.

Editor's comment—*This interesting paper suggests that treatment for excessively tall stature in healthy girls should be initiated prior to mid-puberty if maximal benefit is to be attained. In addition, the finding that high-dose estrogen works primarily at epiphyseal growth centers leads to speculation about the use of estrogen therapy in agonadal girls. It would be of interest to evaluate the effect of low-dose estrogen on lower limb length and growth velocity in normal girls and in girls with Turner syndrome, particularly because others have suggested that sex steroids play no role in the growth rate of agonadal children prior to puberty. Similarly, the effects of low- and high-dose testosterone therapy in agonadal and constitutionally delayed-growth boys should also be pursued (with knemometry).*

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A Preliminary Report on the Role of Somatostatin Analog (SMS 201-995) in the Management of Children with Tall Stature

This paper presents preliminary results obtained in seven children with excessive height and height velocity leading to a height prediction (TW2 method) >180 cm in girls (n=5) and >200 cm in boys (n = 2) treated for more than 6 months with one daily injection of the long-acting somatostatin analog SMS 201-995. Two of the participants were prepubertal, two at pubertal stage 2-3, and three at stage 4.

Growth hormone (GH) secretion, measured as the sum of the amplitudes of the GH pulses during 24 hours, deeply decreased after the first dose of the analog and was still low following 1 year of therapy in patients who were retested. No change occurred in serum thyroxine levels or in glucose and glycosylated hemoglobin A1C values. A small, nonsignificant decrease of serum insulin values was observed.

Mean growth rate decreased significantly from 8.3 (range, 5.5-12.2) to 3.0 (range, 0.2-4.5) cm/yr at the end of 6 months of treatment. It remained <5 cm/yr after 1 year in the four patients who were still receiving therapy. The effect of the treatment on predictable adult height was measurable in five patients, with a reduction of -2.1 to -6.3 cm in three, and no reduction in two (-1.1 and +0.7 cm), although this predicted height reduction was only borderline significant.

The authors point out, from this preliminary work, that SMS 201-995 effectively reduced the secretion of GH, with no important side effects, during treatment for 6 months to 1 year, but that it also had no constant or significant effects on the predictable adult height. They suggest that SMS 201-995 may have a role in the management of excessively tall children, but the optimum mode and timing for its use remain to be established.

Hindmarsh PC, Pringle PJ, DiSilvio L, Brook CGD. *Clin Endocrinol* 1990;32:83-91.

Editor's comment—*Avoiding excessive adult height remains a challenge, as predictions and results for the treatments that have been proposed remain uncertain. This new approach deserves consideration, mainly because no harmful effects have been observed and the drop in GH secretion has been well documented. Everyone will agree with the authors that the data are preliminary and allow no more than continuing clinical research with somatostatin analogs as a possible treatment for extremely tall children.*

Jean-Claude Job, M.D.

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The Adverse Systemic Effects of Growth Hormone in Acromegaly

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Chronic hypersecretion of growth hormone (GH) in adults results in acromegaly. Growth hormone is secreted in a pulsatile fashion in both normals and acromegalic patients, but whereas GH concentrations remain detectable in serum throughout the 24-hour measurement period in acromegalic patients,[1] in normal subjects GH concentrations decay to undetectable levels several times during a 24-hour period.

A GH-secreting pituitary adenoma is the cause in 99% of cases of acromegaly. Rare causes include eutopic (eg, hypothalamic gangliocytoma) or ectopic (eg, carcinoid or islet cell tumors) hypersecretion of GH-releasing hormone. After epiphyseal fusion, excessive GH causes a gradual enlargement of the acral skeleton, most notably the hands, feet, nose, and mandible. However, these changes occur very slowly and may not become recognizable for 10 to 20 years. In addition to its effects on the skeleton, GH hypersecretion adversely affects most organ systems. In a retrospective review of 194 acromegalic patients treated between 1939 and 1967, Wright and coworkers observed a twofold increase in mortality rates compared to the general population of England and Wales, particularly from cardiovascular, respiratory, and cerebrovascular deaths.[2] This paper will review recent advances in our understanding of the consequences of GH hypersecretion observed in acromegalics.

Endocrine Complications

Insulin resistance induced by GH hypersecretion results in glucose intolerance in 29% to 45% of acromegalic patients and frank diabetes mellitus in 10% to 20%.[3-5] Insulin response to an intravenous glucose challenge is exaggerated in patients with normal glucose tolerance and is decreased and delayed when glucose tolerance is abnormal. In the latter group, lowering GH concentrations usually results in improved, although possibly still abnormal, glucose tolerance.[3] The risk factors for development of diabetes in acromegalics have not been well defined. Higher serum GH levels are associated with a higher incidence of diabetes, but HLA phenotype, family history of diabetes, and duration of acromegaly do not have predictive value.[4,5] Pancreatic islet cell antibodies are negative in diabetes caused by acromegaly.[5]

Hypertriglyceridemia occurs in 19% to 44% of acromegalic patients, especially those with abnormal glucose-insulin response,[6] and improves with reduction of serum GH concentrations. Hepatic triglyceride lipase and lipoprotein lipase activities are decreased; they increase when serum GH levels are lowered.[7] The effect of GH hypersecretion on serum cholesterol concentrations is not clear, as current reports conflict.[6,7]

Hypogonadism in acromegaly may result from either destruction of gonadotrophs by a pituitary tumor or by alterations in gonadotropin releasing hormone secretion as a result of coexistent hyperprolactinemia. Menstrual disorders occur in 32% to 87% of women under 45;

Letter From the Editor

Pediatricians seldom see patients who produce excessive growth hormone (GH). However, as GH is given to children for an increasing number of indications, the signs and symptoms associated with excessive GH become of increasing academic interest. Internists who are endocrinologists deal with the problem of acromegaly frequently, and we have capitalized on their experience. Dr Mark Hartman has very nicely summarized the complications of acromegaly in the article beginning on this page.

For the reader with limited experience in the administration of GH to children, please understand that the complications found in adult acromegaly are highly unlikely to occur in children at the doses of GH currently used.

Robert M. Blizzard, MD

Robinow Syndrome: An Update—See page 6

27% to 46% of men have decreased libido or impotence.[4] Galactorrhea occurs in approximately 20% of women, of which 10% to 28% may have normal serum prolactin concentrations. In the latter case, galactorrhea has been postulated to arise from specificity spillover effects of GH on prolactin receptors.[8]

Thyromegaly is observed on examination in 25% to 53% of acromegalic patients. Thyroid ultrasonography reveals a higher incidence of increased thyroid volume (71%), as well as a high incidence of multiple thyroid nodules (65%). Serum thyroglobulin concentrations are increased in 47% of acromegalics. These effects may be mediated by increased concentrations of insulin-like growth factor I (IGF-I). Thyroid volume and serum thyroglobulin levels decrease with effective therapy of acromegaly.[9]

Calcium and Bone Metabolism

The alterations in calcium metabolism in acromegaly are depicted in Fig 1.[10,11] These changes occur apart from any changes in serum concentrations of parathyroid hormone or calcitonin. The major adverse consequence of hypercalciuria is an increased incidence of urolithiasis, which ranges from 6% to 12.5%.[12] All of these abnormalities reverse when serum GH concentrations are normalized.

Bone formation and resorption, as assessed by quantitative microradiography, are both increased in acromegaly. However, bone formation exceeds resorption in rib cortex, whereas the reverse occurs in the iliac crest. These findings suggest that bone remodeling is increased in acromegaly, with a possible redistribution of bone mass from trabecular to cortical bone.[13] Although bone mineral density (BMD) in the radius is consistently increased in acromegaly, there are conflicting data about the BMD of the vertebral bodies (Table 1).[13-15] However, in the only study that documented decreased spinal BMD, hypogonadism was a confounding factor in half of the patients.[15]

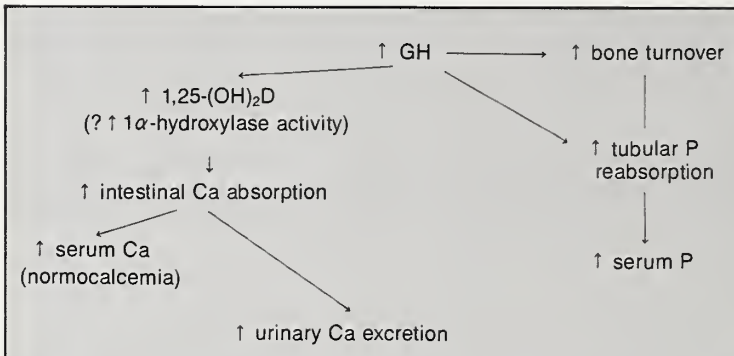

Figure 1—Alterations in calcium metabolism associated with acromegaly.[10,11]

Table 1—Forearm and spinal bone mineral density in acromegaly

Ref	No. of patients	Acromegaly status	Gondal status	Forearm BMD (*P* v ctrl)	Vertebral BMD (*P* v ctrl)
13	26	active	normal	↑ ($P < 0.05$)	ND
14	7	active	normal	↑ (NS)	↑ ($P < 0.01$)
15	12	active	normal	↑ ($P < 0.001$)	↓ (NS)
	5	active	hypo	↑ ($P < 0.05$)	↓ ($P < 0.05$)
	7	inactive	hypo	↓ ($P < 0.01$)	↓ ($P < 0.05$)

BMD, bone mineral density; ND, not done; NS, not statistically significant; ctrl, control subjects.

Gastrointestinal Neoplasms

Table 2 summarizes the findings of 3 studies that have documented an increased incidence of gastrointestinal neoplasms in acromegalic patients, including colonic polyps and adenocarcinoma of the colon and stomach.[16-18] Since adenomatous colonic polyps are considered a premalignant condition, these authors recommend that colonoscopy be performed in acromegalic patients over age 50, especially if more than 6 skin tags are present.

Cardiovascular Complications

Cardiovascular disease is the most common cause of death in acromegalic patients, with mortality rates twofold above those expected for men, but these are not increased in women. This increased mortality is associated with hypertension.[2]

Idiopathic hypertension occurs in 13% to 50% of acromegalic patients. It is associated with higher mean GH concentrations and with longer duration of acromegaly. It is usually mild, uncomplicated, and responds well to a variety of antihypertensive medications.[19]

Sodium retention, expansion of extracellular fluid volume, and suppression of the renin-angiotensin-aldosterone axis are observed in both hypertensive and normotensive acromegalics. Overactivity of the sympathetic nervous system may also be a possible etiologic factor.[20] However, there are conflicting data on whether hypertension improves with therapy of GH hypersecretion.

The existence of a specific type of heart disease in acromegaly is con-

In This Issue

troversial. In a recent retrospective review of 256 consecutive patients, 10 acromegalics were identified with heart disease without evidence of hypertension, diabetes, thyroid disease, or coronary or valvular heart disease. Patients with active acromegaly uniformly developed worsening cardiac function. Among patients cured of acromegaly, equal numbers experienced improvement, stabilization, and deterioration of cardiac function.[21] Pathologic findings in the hearts of acromegalics include myocardial hypertrophy (93%), interstitial fibrosis (85%), and lymphomononuclear myocarditis (59%).[22] Approximately half of acromegalic patients have left ventricular hypertrophy (LVH), and about one quarter have evidence of abnormal left ventricular function, as assessed by echocardiography and radionuclide imaging studies (Table 3). While most of these studies included hypertensive patients, LVH was seen in 43% to 64% of normotensive patients as well.[23-29]

The cardiac complications of acromegaly may improve or stabilize with therapy of GH hypersecretion, although left ventricular function may continue to deteriorate in some patients with long-standing disease.

Skin and Soft Tissue Changes

The earliest manifestations of acromegaly include oily skin, hyperhidrosis, and soft tissue swelling of the hands, fingers, and feet. After successful transsphenoidal surgery, these signs resolve almost immediately. Increased skin thickness results in exaggerated facial wrinkles and nasolabial folds; these resolve more slowly with therapy. Multiple skin tags, increased skin pigmentation, and coarsened and darker scalp and body hair may be seen. Women may have mild hirsutism. The lips, tongue, nose, and ears are enlarged; enlargement of the vocal cords, larynx, and sinuses results in a deeper, more resonant voice. These signs are important since they occur early in the natural history of acromegaly and are reversible with therapy, with the exception of growth of cartilaginous structures.

Acromegalic Arthropathy

Arthralgias are a common presenting complaint of acromegaly, occurring in 62% to 75% of patients. Objective arthropathy is observed in 16% to 62% of patients; 10% to 40% experience arthropathy severe enough to limit activities of daily living. Among peripheral joints, the knees, hips, and shoulders are more frequently affected; the elbows and ankles are less commonly involved. Although the entire spine may be involved, the lumbosacral spine is usually more affected than the cervical or thoracic spine. Subcutaneous thickening of periarticular tissues causes the earliest symptom of joint tightness, especially in the hands. This symptom may resolve with effective therapy of GH hypersecretion. Significant joint pain usually indicates that irreversible cartilage degeneration has occurred.[30,31]

Early in acromegaly, joint spaces are increased, secondary to cartilage proliferation. Synovial and periarticular swellings result in joint swelling without effusion. As cartilage proliferation continues, ulcerations develop at weight-bearing sites, resulting in abnormal joint geometry. New bone formation and remodeling ensues, with development of osteophytes and, ultimately, narrowed joint spaces. The end stage is a debilitating, severe osteoarthritis, which may require artificial joint replacements. There is no evidence for an inflammatory component. In the spine, disc spaces are

Table 2—Gastrointestinal neoplasms in acromegaly

Ref	Study type	No. of pts	No. pts with colonic polyps	No. pts with GI malignancy	O/E ratio for GI malignancy
16	Pr	17	9 (53%)*		
	R	44		4 colon (9.1%)	ND
17	Pr/R	12	4 (33%)**	3 colon (25%)	> 3 ($P < 0.001$)
18	R	48	ND	5 (10%) (3 colon, 2 gastric)	4.6 ($P < 0.05$)

Pts, patients; Pr, prospective; R, retrospective; ND, not done; O/E, observed/expected.
* 8 pts with polypectomies: 5 had adenomatous and 3 had hyperplastic polyps.
** 3 pts with polypectomies: 2 had adenomatous and 1 had hyperplastic polyps.

Table 3—Echocardiographic and radionuclide studies of the heart in acromegalic patients

Ref	No. of patients	LV hypertrophy*	Abnormal LV function**	Correlation with GH
23	10	ND	7 (70%)	–
24	16	7 (44%)	6 (38%)	+
25	23	13 (57%)	4 (17%)	+
26	25	20 (80%)	3 (12%)	+
27	27	14 (52%)	6 (22%)	+
28	16	6 (38%)	3 (19%)	ND
Total	117	60/107 (56%)	29/117 (25%)	

ND, not done; LV, left ventricular.
* Increased LV mass or wall thickness, concentric LVH, asymmetric septal hypertrophy.
** Abnormal systolic time intervals, systolic shortening fractions, ejection fractions.

Erratum

In *Growth, Genetics, and Hormones* Vol. 6, No. 1 (March 1990), an error on page 14 incorrectly linked neural tube defects with a *low* alpha-fetoprotein level found in maternal serum screening. In fact, neural tube defects are associated with a *high* maternal alpha-fetoprotein level.

increased and dorsal kyphosis and anterior osteophytes are common. Although patients frequently complain of backaches, spinal mobility is usually normal or increased, apparently because the discs remain resilient and the paraspinal ligaments become hypertrophied and somewhat lax.[30,31]

Results of 2 recent studies suggest that arthropathy is worse in patients with long duration of acromegaly.[32,33] These reports also indicate that therapy is most likely to improve joint symptoms if it is initiated early in the course of the disease and if serum GH concentrations are rapidly lowered. If GH levels remain elevated for many years, irreversible cartilage degeneration occurs, and the arthropathy is less likely to respond to therapy.

Neuromuscular Complications

Carpal tunnel syndrome occurs in 30% to 64% of patients with acromegaly. Symptoms include paresthesias in the median nerve distribution and hand pain at night or with prolonged use. On physical examination, a positive Tinel's sign or Phalen's sign, or thenar muscle atrophy strongly suggests the diagnosis. A nerve conduction study may be helpful to confirm the diagnosis. Hyperplasia of ligaments and tendons in the carpal tunnel with synovial edema lead to compression of the median nerve. The syndrome usually resolves within 6 weeks of normalization of serum GH concentrations.[34] In 1 study, electroneurographical findings improved within 1 week of transsphenoidal surgery in 12 of 28 patients (43%).[35]

Table 4 outlines the clinical features of the proximal myopathy of acromegaly. Muscle biopsies in these patients have revealed hypertrophy of type I and II muscle fibers, muscle fiber necrosis, an increased number of sarcolemmal nuclei, and increased glycogen and lipofuscin deposits. Proximal myopathy is associated with a longer duration of acromegaly and improves very slowly after reduction of serum GH concentrations.[34,36]

Respiratory Complications

Respiratory mortality rates are increased threefold in acromegalic patients.[2] Two studies have reported that abnormal pulmonary function tests correlated with the duration of acromegaly.[37,38] Whether any of these changes are reversed by successful therapy is unknown.

Three major clinical presentations of upper airway problems occur in acromegalic patients. The most unusual is an acute exacerbation of upper airway narrowing by an upper respiratory infection, resulting in the acute onset of dyspnea and inspiratory stridor. A flow-volume loop study may be helpful in making the diagnosis.

A second, more common syndrome is difficulty with intubation. Careful preoperative assessment, the use of fiberoptic laryngoscopy, and the "sniffing position" may facilitate intubation in these patients. Occasionally, tracheostomy may be required. In all cases, the patient should be closely monitored following extubation for the development of upper airway obstruction.

The third and most recently recognized manifestation of upper airway narrowing in acromegalics is the obstructive sleep apnea syndrome (OSAS). Symptoms include excessive daytime sleepiness, habitual snoring, restless nocturnal sleep, and apneic episodes. Polysomnography is required for diagnosis since pulmonary function tests, including flow-volume loops, may be normal in these patients. The diagnosis is made when more than 5 apneas occur per hour of sleep. The prevalence of OSAS in 3 series was 40% to 50% and 0% of patients with active and long-standing inactive disease, respectively (Table 5).[39-41] Men were affected more commonly than women. Both the prolapse of an enlarged tongue and the inspiratory collapse of the hypopharynx have been implicated by endoscopic studies.[42,43] Short-term studies in a limited number of patients have indicated that lowering GH levels results in improvement or resolution of OSAS in approximately 50% of patients after 6 days to 12 months of follow-up. However, OSAS may persist in some patients whose serum GH levels have been normalized by successful therapy, at least for periods up to 1 year. (Table 6).[41,43-45] These preliminary observations suggest that the reversibility of obstructive sleep apnea in acromegaly may depend on which anatomical structures are most affected in a given patient. For patients in whom sleep apnea persists despite therapy, effective treatments include nasal continuous positive airway pressure (CPAP) at night, surgical reduction of the tongue and/or other pharyngeal tissue, and tracheostomy.

Conclusions

Excessive GH secretion in acromegaly adversely affects most organ systems, leading to increased morbidity and mortality. As many of these complications correlate with the duration of acromegaly, it is important to make the diagnosis early in the course of the disease and to initiate therapy to rapidly decrease serum GH concentrations to the normal range. The measurement of serum IGF-I concentration is a sensitive screening test for

Table 4—Proximal myopathy in acromegaly

Ref	No. of patients	History	Weakness PE	CPK or aldolase	Abnormal EMG	Abnormal biopsy
34	17	76%	41%	18%	46%	1/3
36	11	55%	55%	45%	*	5/9

CPK, creatine phosphokinase; EMG, electromyography; PE, physical exam.
* Mean action potential duration significantly decreased compared to control subjects.

acromegaly. The diagnosis may be confirmed if the serum GH concentration is greater than 2 μg/L, 60 minutes after a 100-g oral glucose load. Random serum GH levels are not helpful, as GH is secreted in a pulsatile fashion in normal subjects. Transsphenoidal surgery by a neurosurgeon experienced in pituitary surgery is the initial treatment of choice, since it will rapidly decrease serum GH concentrations. If serum GH levels are not normalized by surgery, therapeutic options include pituitary irradiation and medical therapy with either octreotide (a somatostatin analogue) or bromocriptine.

Table 5—Prevalence of the obstructive sleep apnea syndrome in acromegaly

Ref	No. of patients	Acromegaly status*	No. with OSAS	Mean serum GH concentration (ng/mL)
39	6	active	3 (50%)	21 ± 18
	5	inactive	0	2.7 ± 1.5
40	10	active	4 (40%)	62
	11	inactive	0	3.2 ± 2.2
41	11	active	5 (45%)	41 ± 38

OSAS, obstructive sleep apnea syndrome.
* Acromegaly was defined as inactive by the authors if the fasting serum GH concentration was <5 ng/mL.

Table 6—Effect of treatment of acromegaly on the obstructive sleep apnea syndrome

Ref	No. of patients	Treatment	No. OSAS resolved	No. OSAS improved	No. OSAS unchanged	No. with normal GH but OSAS unresolved
41	5	TSS	1	1	3	2
43	1	TSS	0	0	1	1
44	1	SMS	0	1	0	1
45	1	BC	0	1	0	0
Total	8		1 (12%)	3 (38%)	4 (50%)	4 (50%)

OSAS, obstructive sleep apnea syndrome; TSS, transsphenoidal surgery; SMS, SMS 201-995 (a somatostatin analogue); BC, bromocriptine.

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Robinow Syndrome: An Update

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In 1969, Silverman, Smith, and I reported a family consisting of 3 siblings, mother, and grandmother with "a previously unrecognized dwarfing syndrome."[1] The major features were: moderately short stature, characteristic facial dysmorphism, genital hypoplasia, and mesomelic brachymelia. The facial characteristics (Fig 1) included hypertelorism; short, upturned nose; long philtrum; broad, triangular mouth; and dental malalignment (Fig 2). The genital hypoplasia consisted of micropenis in the males (Fig 3) and hypoplastic clitoris and labia minora in the females. Somewhat later, I proposed the more descriptive term "fetal face syndrome," since the face resembles that of the human fetus at 8 weeks[2] (Fig 4).

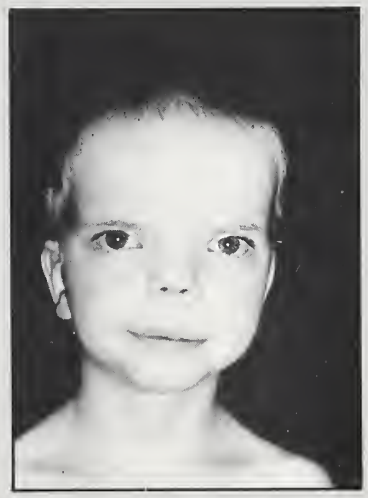

Figure 1—Index patient (dominant type). Face at 7 years. Large forehead, hypertelorism, short upturned nose, long philtrum, broad mouth.

In 1973, Wadlington et al[3] published 4 more cases of the syndrome and added vertebral segmentation anomalies to the clinical spectrum. In the more than 30 publications on the syndrome,[4] a number of other, less constant anomalies have been described, some nonspecific, others more nearly unique and thus of greater diagnostic value (Table).

In most cases, the diagnosis is obvious on inspection. In "atypical" cases, when some of the major features are missing, the diagnosis must remain in doubt. Atypical cases have not been reported in relatives of "classic" cases.

Genetics

In the index family,[1] transmission of the syndrome was autosomal dominant. Two of the patients of Wadlington et al,[3] boy-girl siblings, were children of normal parents, strongly suggesting autosomal recessive inheritance. Since then, both modes of inheritance have been amply documented. As to be expected, pedigrees indicating recessive inheritance have been encountered more often in populations with high rates of consanguinity, eg, Arabs.

I believe characteristic dominant and recessive phenotypes can be delineated.[5] Individuals with the dominant form seem to have normal stature or only mild dwarfing, no vertebral abnormalities or, at most, a single butterfly vertebra, and only mild forearm brachymelia. Patients with the recessive form seem to have more severe dwarfing, more extensive vertebral segmentation defects, and more severe forearm brachymelia and dysplasia (Fig 5). Intrafamilial variability has been slight while interfamilial variability has been considerable, so that further genetic heterogeneity seems likely.

Unfortunately, all the diagnostic criteria for this syndrome are morphologic. No metabolic defect has been identified; no animal model is known. Chromosome studies have yielded uniformly normal results, although chromosome mapping has not been attempted.

Teratogenic Period

Since the face and the external genitalia attain their final shape at around 10 weeks of gestation, some authors have speculated that this may also be the teratogenic period

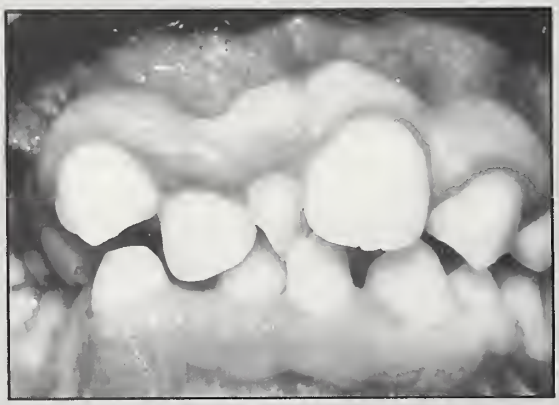

Figure 2—Same patient. Dental malalignment and gingival hyperplasia.

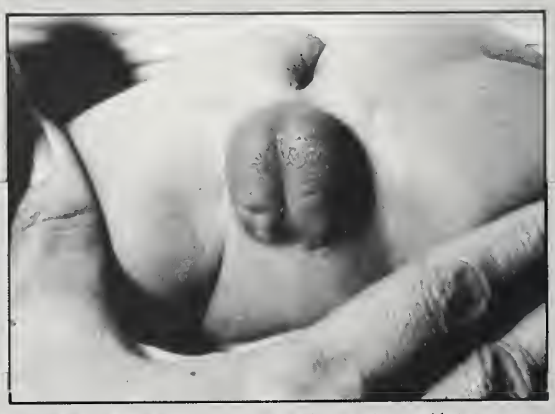

Figure 3—Extreme micropenis in newborn. Note normal-sized scrotum and testicles.

Table—Anomalies in Robinow syndrome

Nonspecific Abnormalities	Syndrome-specific Abnormalities
Cryptorchidism	Midline indentation of the lower lip with tongue tie and bilobed tongue tip
Inguinal hernia	Gingival hyperplasia (Fig 2)
Hypospadias	Partial or complete duplication of distal phalanx of thumb or big toe
Congenital heart disease	Distal ulnar and proximal radial hypoplasia with proximal radio-ulnar dislocation (Fig 5)
Cleft lip/palate	Apparent exophthalmus due to hypoplasia of the lower lids
Brachydactyly, clinodactyly	
Mental retardation (rare)	

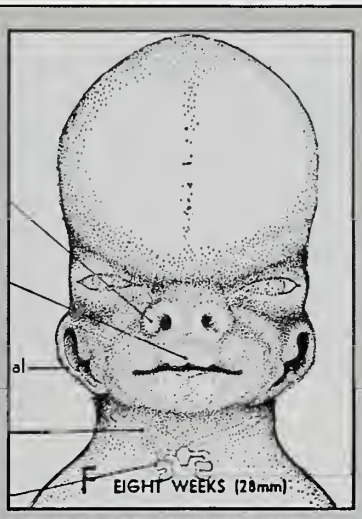

Figure 4—The "fetal face." Human embryo at 8 weeks.[2] Reproduced with permission of McGraw-Hill Book Co.

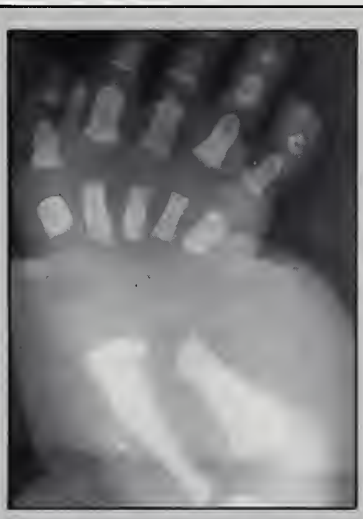

Figure 5—Autosomal recessive type. Severe acromesomelic brachymelia, short metacarpals and phalanges, hypoplastic distal ulna and proximal radius, and radio-ulnar dislocation.

for the syndrome. However, vertebral segmentation is normally completed at 4 weeks, suggesting that teratogenesis is more extended, at least in the recessive type.

Course

Approximately 10% of patients have died in infancy, most of them of pulmonary disease or cardiac malformations. Probably all the deaths occurred in the recessive type. The remaining 90% seem to have enjoyed good health. The facial features become less striking at puberty, which may explain the fact that almost all index cases have been in infants and young children.

Endocrine Aspects

Sexual maturation occurs at the usual age in both sexes. In males, the penis remains abnormally short but may attain normal circumference, permitting sexual function. Endocrine studies by Lee et al[6] suggested partial primary hypogonadism. Androgen receptors and 5α-reductase in genital skin fibroblasts were normal. Females with both the dominant and recessive forms have reproduced, as have males with the dominant type. Reproduction by males with the recessive form has not yet been documented.

The Future

Further studies of the phenotype are not likely to add much to present understanding. Progress will have to await metabolic and molecular genetic studies. Once the gene abnormality(ies) has (have) been identified, we can derive a better classification and may gain a better insight not only into the teratogenesis of the syndrome but also into mechanisms of normal embryogenesis.

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Phenotype Abnormalities Seen with 45X/46XY Mosaicism

With the advent of prenatal diagnosis, 45X/46XY mosaicism is ascertained on a fairly regular basis. The question is whether 45X/46XY mosaicism is associated with Turner syndrome, infertility, ambiguous genitalia, or any other problems. Until recently, selection for testing for 45X/46XY mosaicism has been based on the presence of unusual postnatal features.

The authors have taken advantage of current prenatal testing procedures to conduct an unbiased survey of 45X/46XY incidence by sending a questionnaire to an international sample of 730 cytogenetic laboratories. A total of 92 cases of prenatal diagnosis of 45X/46XY mosaicism were reported. There was good clinical information on 76 cases; 75 were phenotypically male and 1 was female. Three of the phenotypic males had hypospadias, and the phenotypic female had clitoromegaly. Many of the cases had been terminated prenatally at the parents' request, and gonad histology was done in 11 cases. Of these, 3 (27%) had abnormal testicular development, but only 1 had abnormal external genitalia. Of the 75 "males," 5 had other congenital abnormalities of consequence. The authors found no relationship between the degree of mosaicism observed at prenatal diagnosis and the severity of abnormalities.

The percentage of 45X cells ranged from 1% to 98%; the majority had less than 50% 45X cells and (probably for this reason) presented with a normal male phenotype rather than a Turner phenotype.

Long-term follow-up of 45X/46XY patients is not available, and information concerning long-term stature, pubertal development, tumor risk, and fertility is needed. However, this study suggests that most patients with 45X/46XY karyotype (95%) have normal male genitalia, in contrast with previous postnatal studies. Dysgenetic gonads appear to occur in about 25% of cases, but whether this figure would be higher by puberty is not yet known. Dysgenetic gonads in normal-appearing males who have never had chromosome studies may be a source of infertility or gonadoblastomas in the general population.

Chang HJ, et al. *Am J Hum Genet* 1990;46:156–167.

Editor's Comment—*This is an important study in view of the frequent use of prenatal diagnosis and concomitant finding of 45X/46XY karyotypes. This study suggests that most cases will do well, but clearly long-term follow-up is needed. Also of interest, it appears that approximately half of the families where this diagnosis was made prenatally have terminated the fetus. These decisions were probably based on expectations of poor outcome ensuing from previous, biased, reports, whereas the actual prognosis may not be so unfavorable for such children.*

Judith G. Hall, MD

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Obesity in Childhood and Adolescence, Part II: Pathophysiology, Associations, and Complications
by W.H. Dietz, MD, and L.G. Bandini, MD

The Polymerase Chain Reaction

During the last 4 years, the technique of polymerase chain reaction (PCR) has revolutionized the way in which molecular genetics is done. PCR is a relatively simple method to amplify or increase the number of copies of a specific segment of DNA, to obtain sufficient DNA for further evaluation. The segment of DNA to be amplified may come from many sources or from DNA in an intact cell. It is possible to amplify a short segment, 50 to over 2,000 base pairs in length, to more than a million copies in just a few hours. Furthermore, the process has been automated and is presently being used for detecting DNA on fixed pathologic specimens, from single cells (lymphocytes, sperm cells, skin cells, etc), from forensic material such as hairs or blood cells, from ancient archeological specimens, and for many molecular genetic studies and diagnostic tests.

The basis of the technique, developed by the Cetus Corp, lies in targeting the DNA segment to be amplified by identifying its boundaries with 2 single-stranded oligonucleotide primers. A heat-stable DNA polymerase is used to catalyze the duplication reaction. The native double-stranded DNA to be amplified is denatured by heat, and once the DNA has been liberated as single strands it can then be duplicated by the polymerase using the primers. Thus, the process moves very rapidly as new copies become templates for more copies. By rapidly alternating the temperature—causing separation of the double strand, allowing duplication—large amounts of double-stranded DNA of the specific short segment are produced.

The technique has endless research applications, including the study of specific mutations, use in genomic cloning, analysis of protein-DNA interactions, a variety of genetic therapies, rapid diagnosis (both prenatal and prior to implantation), unique identification of tissues, diagnosis of infectious states such as HIV, etc; and there will undoubtedly be additional major applications in the years to come.

Eisenstein BI. *N Engl J Med* 1990; 332:178–183.

Editor's Comment—*This is an excellent review. All individuals in medicine should understand the PCR technique; if you don't, read the article!*

Judith G. Hall, MD

Nontraditional Inheritance: Genomic Imprinting in Glomus Body Tumors

The most striking finding in this report was that the familial form of this disorder *is transmitted by males only*, either directly or through unaffected females, but *is not an X-linked condition*. Interestingly, these tumors are very much like other familial tumors, in that when they are familial, they are of early onset, occur at multiple sites, and have more severe symptoms and arise at a younger age than nonfamilial tumors.

This recent review by van der Mey et al of patients with glomus tumors identified 69 patients, of whom 34 had no family history and 35 had a family history. Another 82 patients were found within the families of the familial cases. Overall, there was a female excess, but this was entirely in those patients without a family history. This was not an X-linked condition because there was male-to-male transmission. The number of affected males and females in the familial cases was the same. The most appealing explanation for these observations is *genomic imprinting*, in which a condition expresses itself only when inherited from a parent of one sex. The maternally derived gene is apparently inactivated during oogenesis in the mother but can be reactivated during spermatogenesis in her male offspring. In the case of glomus tumors, the family history suggests that when the gene is inherited from the mother, the genetic information is somehow suppressed; but when inheritance is from the father, the genetic information allows expression of the tumors over time. These are slow growing, benign, single or multiple tumors. They are known as chemodectomas or nonchromaffin paragangliomas and are derived from the glomus body tissue. They are most often found in the carotid body but also sometimes in the adventitia of the carotid bifurcation, the glomus jugulare, or the vagal body. Genomic imprinting seems to be common among familial and congenital tumors.

van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ, et al. Genomic imprinting in hereditary glomus tumors: evidence for a new genetic theory. *Lancet* 1989; 2:1291–1294.

Editor's Comment—*The concept of genomic imprinting is important and exciting. It may explain patterns of inheritance that have not previously been easily understood. Reexamination of a large pedigree, looking for differences in expression when the disorder is inherited from the mother versus the father, presents a whole new way of looking at information and understanding mechanisms of genetic expression.*

Judith G. Hall, MD

Steroids and Bowel Rest Versus Elemental Diet in the Treatment of Patients With Crohn's Disease: The Effects of Protein Metabolism and Immune Function

The traditional management of acute attacks of inflammatory bowel disease (Truelove regimen) consists of bowel rest, intravenous fluids, steroids, and antibiotics. However, there have been studies suggesting that an elemental diet is as effective as steroids in inducing remission from an acute attack of Crohn's disease. This study was undertaken to investigate the metabolic and immunological effects of these 2 disparate therapies.

Six patients with chronic Crohn's disease who met the inclusion criteria of a palpable inflammatory mass, elevated erythrocyte sedimentation rate (ESR), nausea, abdominal cramps, weight loss, and absence of obstruction were randomly assigned to receive a 1-week course of either steroids (400 mg/dL) plus bowel rest and intravenous fluids, or an elemental diet alone. At full strength, the elemental formula (Elental ED), infused via a nasogastric tube, provided 2,000 calories from glucose polymers, MCT oil, and 84 g of amino acids. Amino acid and protein turnover ratios, assessed by ^{14}C-labeled tracers, plus immunological status were assessed initially and again on day 7 of treatment. Total nitrogen losses were estimated by adding 2 g of nitrogen to the 24-hour nitrogen excretion.

Clinical and symptomatic improvement occurred in all patients. Improvement was also reflected by more normal ESR values, platelet counts, and serum albumin and globulin concentrations. The steroid therapy resulted in higher levels of glucose, insulin, and cortisol, but lower T-lymphocyte counts, immunoglobulin concentrations, and IgG synthesis rates. Plasma amino acid concentration, protein breakdown, and albumin synthesis increased in the steroid-treated patients whereas they fell in the patients who received the elemental formula. Nitrogen excretion increased in both groups over the duration of the study, but the mean nitrogen balance on day 7 was +2.4 g/d for the group receiving the elemental diet and −8.9 g/d for those who received the steroid regimen. Both therapies were associated with increased rates of plasma amino acid flux, amino acid oxidation, whole body protein turnover, and suppressed lymphocyte subsets, lymphocyte transformation, and serum complement concentrations.

The authors conclude that the primary difference with the steroid therapy was greater immunosuppression and higher nitrogen loss.

O'Keefe SJD, Ogden J, Rund J, et al. *J Parent & Ent Nutr* 1989; 13:455–460.

Editor's Comment—*This study is very important; it is the first well-designed scientific study that attempts to evaluate the effects of bowel rest with steroid therapy versus an elemental diet for the management of an acute attack of Crohn's disease. In this prospective, blinded study, the authors demonstrate that both treatments induced clinical and symptomatic improvement of the disease; however, the treatment with steroids plus bowel rest was associated with greater immunosuppression and a more severe loss of nitrogen than occurred with the elemental diet. With the steroid-based therapy there was a cumulative loss of 55 g of body nitrogen (equivalent to 360 g of protein or 1.5 kg of lean body mass). This high catabolic state persisted even after the disease was in remission, suggesting that steroids may interfere with normal adaptation to fasting. In contrast, an elemental diet reversed this process and resulted in a small gain in body nitrogen.*

The data clearly show that from a nutritional point of view, it is difficult to support the use of a starvation regimen in patients with acute disease. Additionally, the data point to a possible role of dietary proteins in the perpetuation of the inflammatory process of patients with Crohn's disease. For the individual patient, the Truelove regimen of bowel rest, steroids, and antibiotics should always be complemented at least with IV nutrition or preferably with an elemental diet. The prompt reversal of the negative nitrogen balance with an elemental diet during acute relapses of the disease may allow children with Crohn's disease to sustain more normal growth. Therefore, I agree with the authors that diet restriction is contraindicated and that there are times when it may be advantageous to avoid the use of steroids.

Fima Lifshitz, MD

Germ-line Mosaicism in Osteogenesis Imperfecta

Germ-line mosaicism is the presence of more than 1 population of germ cells within a gonad. It is suspected when multiple children affected with an autosomal dominant disorder or a disorder that results from a new mutation of an X-linked gene are born to normal parents. Although evidence for such mosaicism is usually circumstantial, Cohn et al document the phenomenon in a family with lethal osteogenesis imperfecta (OI) type II. Two affected sons were born to an "unaffected" father by 2 separate wives. Electrophoretic abnormalities typical of OI type II were detected in type I collagen synthesized by skin fibroblasts from both affected infants, but not from the father or from 2 unaffected sisters of the second son. Further analysis pointed to an abnormality in the $\alpha 1$(I) collagen chain; ultimately, a single nucleotide change resulting in a substitution of aspartic acid for glycine at position 883 of the triple helix was detected. Since the base change disrupted a restriction endonuclease cleavage

site, it allowed the normal gene to be distinguished from the mutant gene, which was exploited to search for the mutation in the germ cells and somatic cells of the father.

A small (225 base pair) fragment containing the exon harboring the mutation was amplified by polymerase chain reaction from genomic DNA isolated from the father's sperm, white blood cells, and hair root bulbs. The mutation was found in approximately 12% of sperm and in about 40% of the somatic cells. Thus, in addition to germ-line mosaicism, the father exhibited somatic mosaicism for the mutation, despite being clinically unaffected. The authors mention that they are aware of several other cases of undocumented germ-line mosaicism in OI type II and point out that it appears to be more common in OI type II (estimated 6% to 7%) than in most other genetic conditions. They conclude that the clinical phenotypes produced in genetic disorders reflect not only the qualitative effects of the mutation but also quantitative effects determined by the abundance and distribution of the cells expressing the mutation.

Cohn DH, Starman BJ, Blumberg B, et al. Recurrence of lethal osteogenesis imperfecta due to paternal mosaicism for a dominant mutation in a human type I collagen gene (COL1A1). *Am J Hum Genet* 1990; 46:591–601.

Editor's Comment—*Determining recurrence risks for Mendelian (single gene) disorders, such as OI type II, used to be simple and straightforward. Standard risk figures are given in any genetics textbook. However, there are a growing number of phenomena that complicate such calculations. Germ-line mosaicism is a good example. In the past, the father in the above case would have been given a negligible recurrence risk considering his normal clinical phenotype and especially his normal collagen electrophoretic studies. However, as demonstrated, his actual risk was substantially higher. Uniparental disomy, in which a child receives 2 copies of a particular chromosome from 1 parent and none from the other, and genomic imprinting, in which the expression of a mutation (and the disease phenotype) is influenced by which parent transmitted the mutation, are 2 other examples. It seems likely that more will be heard about these phenomena that distort Mendelian risk figures as their investigation receives more attention.*

William A. Horton, MD

Natural History of Premature Thelarche in Olmsted County, Minnesota, 1940 to 1984

Van Winter et al report a population-based study of the incidence of premature breast development in girls between the ages of 6 months and 6 years in Olmsted County, Minnesota, for 1940 to 1984. Because of the dossier-type recording by the Mayo Clinic and other health providers in this community, diagnoses are indexed so that the details of medical care for the entire community are available for review. The authors identify cases of unilateral or bilateral benign breast development occurring between the ages of 6 months and 8 years if other signs of sexual maturity had not developed by 8 years of age. A total of 66 girls were identified, for an incidence rate of 21.2 per 10^5 patient-years; 48 of these had early breast development as an isolated finding and 43 of the 48 could be followed through age 8. Of the 48 girls, 23 had bilateral breast development ranging from 1 to 6.5 cm in diameter. Of the 48, 43 were located and 39 responded to a survey concerning the development of early puberty, breast cancer, gynecologic malignancy, or autoimmune disease. Of 25 respondents between the ages of 16 and 42 years, all had attained an adult height between 155 and 173 cm, their mean age of menarche was 12.6 years, and 10 women had attempted pregnancy and conceived.

The authors point out that this is the first population-based study to show that premature thelarche is self-limiting and has a low incidence. In most of the patients, the premature thelarche disappeared before the onset of puberty and was followed by normal puberty, including menarche and normal reproduction.

Van Winter JT, Noller KL, Zimmerman D, et al. *J Pediatrics* 1990; 116:278–280.

Editor's Comment—*Although there have been reports of the prevalence of premature thelarche, there have been no studies that systematically followed these girls through puberty, young adulthood, and the reproductive years. There have been various reports in the literature of clusters of cases of premature thelarche, but as the authors point out, their significance is unclear because it is unknown whether the observed cases were more numerous than might have been expected by chance alone. The Olmsted County, Minnesota, and Mayo Clinic data provide a unique opportunity to perform such a population-based study. However, the causes of thelarche may be multiple. In addition, epidemics do occur, as for example those reported in Puerto Rico. Therefore, the incidence of thelarche will vary from geographic site to geographic site, and possibly from year to year. For example, between 1940 and 1960, estrogens were frequently found in vitamins, meats, and other ingestible products, but no ingestion of contaminating estrogens has been found in the patients in Puerto Rico* (J Pediatr *1985;107:393–396). While*

this short paper is an important addition to our understanding of the benign course of this disease, the statistics regarding incidence must be regarded as applying only to Olmsted County, Minnesota.

William L. Clarke, MD

Growth in Hemophilic Boys After HIV Infection

Pasi et al measured height and weight 3 times yearly in 26 boys with hemophilia A who became HIV positive during the period from 1981 to 1986. Ten of the boys presently have AIDS-related complex. Height and weight recordings were analyzed over a mean period of 9.2 years, with a mean duration of HIV seroconversion of 4.5 years. Mean growth (height and weight) before and after seroconversion were analyzed in this group by the Wilcoxon matched pairs signed rank test. No significant change in growth or weight was observed after HIV seroconversion. One boy who developed clinical AIDS continued to grow along his respective percentile, and 1 boy with constitutional short stature continued to grow along his respective percentile. Only 1 boy failed to grow along the original percentile, but his growth retardation began 3 years before HIV seroconversion.

Pasi KJ, Collins MA, Ewer AK, et al. *Arch Dis Child* 1990;65:115–118.

***Editor's Comment**—This short descriptive paper is the first to document growth in children with asymptomatic chronic HIV infection. As noted by the author, growth failure has been described previously in children with chronic symptomatic HIV infection. The preservation of linear growth in the present sample (up to 6 years) demonstrates the heterogeneity of the complications seen in this syndrome.*

William L. Clarke, MD

Increase in Serum Concentration of Keratan Sulfate After Treatment of Growth Hormone Deficiency With Growth Hormone

Pachman et al measured the serum concentrations of keratan sulfate (KS) in 2 groups of children with short stature: 1 group with constitutional delay and the other with growth hormone deficiency (GHD). The study populations consisted of 14 children between 8 and 11 years of age with constitutional delay and 9 children, ages 8 to 15, with GHD, defined as a peak GH $\leq$ 10 ng/mL with insulin-induced hypoglycemia, oral L-dopa, or glucagon. The GHD children were growing at a rate $<$4 cm/yr whereas the children with constitutional delay were growing $>$5 cm/yr, which was nevertheless below the fifth percentile.

In children with constitutional delay, KS averaged 414 $\pm$ 118 ng/mL, compared with 505 $\pm$ 126 ng/mL in children from a control population (which consisted of 33 children 8 to 11 years old with normal growth). In the GHD children, KS levels were determined at the time of initial evaluation and after 3 to 15 months of GH therapy. These levels initially ranged from 239 to 587 ng/mL, encompassing the levels in the children with constitutional delay. However, 7 of the 9 children with GHD had a rise in KS ranging from 64 to 192 ng/mL during GH therapy. This increase in KS was correlated with an increase in growth velocity.

The authors point out that KS is a glycosaminoglycan that is almost exclusively derived from the metabolism of cartilage proteoglycans and that the amount of KS in the blood is directly proportional to the rate of degradation of cartilage proteoglycans. They previously reported that serum levels of KS rise from a low level in infancy to reach a plateau by age 4 to 5 years. The measurement of serum KS is felt to be an indicator of the response of chrondrocytes to IGF-I. The relationship demonstrated between increased growth and increased serum KS suggests that KS may be a reasonable indicator of cartilage proteoglycan metabolism during growth. The authors note that 2 patients with GHD who did not increase their KS levels after GH therapy, despite increases in growth velocity, had pretreatment KS levels at the upper range of normal for age.

Pachman LM, Green OC, Lenz ME, et al. *J Pediatrics* 1990;116:400–403.

***Editor's Comment**—Measurement of keratan sulfate (KS) may be a useful indicator of the activity of GH/IGF-I in bone metabolism. These data are somewhat confusing, however, as the increase in KS was relatively modest despite marked increases in growth velocity in the GHD children on GH therapy. This may be due to the heterogeneity of the pretreatment KS levels in this group of children and also to the fact that KS levels plateau in early childhood. The authors correctly point out that an increase in KS indicates a change in the metabolism of proteoglycans, but it cannot be used to predict changes in growth velocity with GH therapy. It is important to remember that IGF-I levels also do not always correlate with response to GH therapy. Nevertheless, it is both interesting and useful to evaluate metabolic changes in bone as a consequence of GH therapy in our attempts to gain a better understanding of how children grow.*

William L. Clarke, MD

Characterization of Dimeric Forms of Human Pituitary Growth Hormone by Bioassay, Radioreceptor Assay, and Radioimmunoassay

Seven highly purified dimeric forms of human pituitary (extracted) growth hormone (hGH) were characterized

from the monomeric forms of 20-, 22-, and 24-kilodalton (kDa) hGH linked together by covalent or non-covalent bonds. Each was studied using 3 different assays: (1) a solid-phase radioimmunoassay (RIA) with rabbit anti-hGH antiserum, the results being expressed in mIU/L by reference to the WHO First IRP 66/217; (2) a radioreceptor assay (RRA) on solubilized bovine liver receptors; and (3) a bioassay (BA) measuring the growth effect on culture of Nb2 lymphoma cells.

These assays produced strikingly different results. In the RIA, all dose/response curves were parallel, except those of the 20-kDa monomeric and the 20/20-kDa dimeric forms. In the RRA, considerable differences appeared in the ability to displace labeled monomeric recombinant hGH from its ligand, with maximal effectiveness for 2 isomers derived from the 22-kDa hGH. The mitogenic effect in the BA was maximal with a non-acidic 20/22-kDa dimer, and minimal with the 20/20-kDa dimer, all the regression lines (number of cells versus log of hormone concentration) being parallel.

Brostedt P, Luthman M, Wide L, et al. *Acta Endocrinol* 1990;122: 241–248.

Editor's Comment—*The general sense of the study is that the various molecular forms of GH found in the pituitary—both monomeric (little) and dimeric (big)—have different mobilities in the 3 types of assays used. It is likely that similar observations would be made for the circulating forms of GH. The authors also note that some variants of hGH occur in the pituitary, mainly in dimeric forms. We may conclude from this that measuring hGH is difficult; that the various types of assays may give discrepant results, depending on the molecular forms of the hormone; and that bioassay with Nb2 cells may be relevant for clinical studies.*

Jean-Claude Job, MD

Insulin-like Growth Factors I and II in Healthy Man: Estimations of Half-Lives and Production Rates

The authors measured the half-life of insulin-like growth factors (IGFs) in 2 normal young adult males after a bolus injection of radio-iodinated IGF-I and IGF-II, with measurement of the serum levels of both the free IGFs and the IGFs bound to their specific carrier proteins. They found a half-life of 10 to 12 minutes for free-labelled IGF-I and -II, 20 to 30 minutes for the 50-kDa bound complex, and 12 to 15 hours for the 200-kDa complex.

In a second step of the study, they infused recombinant IGF-I, 20 μg/kg per hour intravenously, during 6 days in the same subjects and measured the different circulating forms of IGFs by RIA after chromatographic separation. By this means, the calculated production rates were found to be 10 mg/d for IGF-I and 13 mg/d for IGF-II.

This agrees with the earlier findings, by the same group and by others, that the 200-kDa complex contains the major pool of IGF in human serum, and confirms that this complex is mainly responsible for the relatively long half-life of IGF in humans. It suggests that, besides the main pool of 200-kDa, the free and the 50-kDa IGF pools, which have a rapid turnover and could account for daily IGF production, are the source of a shift toward the 200-kDa pool.

Guler HP, Zapf J, Schmid C, et al. *Acta Endocrinol* 1989;121:753–758.

Editor's Comment—*These physiological data in adult humans are possibly of great importance for the interpretation of measurements of IGFs, mainly of IGF-I, in growing children and adolescents. Probably measurement of free IGF-I and of the 2 main IGF-I carrier protein complexes could reduce the difficulty in correlating the results of routine IGF-I assays with such clinical data as height or growth rate.*

Jean-Claude Job, MD

Comparison of Education and Occupation of Adults With Achondroplasia With Same-Sex Sibs

A common concern to parents of children with achondroplasia is that the children will suffer occupational discrimination when they grow up. To investigate the issue, Roizen and colleagues compared education and occupation levels in adults with achondroplasia to those of same-sex sibs. Information was gathered by interview or questionnaire from 8 affected men and 32 unaffected brothers and from 12 affected women and 35 unaffected sisters. An occupational score was calculated from a subscale of the Hollingshead Four Factor Index of Social Status. No significant differences in age or education were noted between the patients and their same-sex sib. The occupation score for affected men was not statistically different from that of their brothers; however, the score for affected women was significantly lower than that of their unaffected sisters. Education level was the single most important variable affecting occupation level for both sexes. The authors speculate that physical deformity accompanying achondroplasia (eg, large head size) may be more detrimental in the workplace to women than to men. They stress the need for more research in this area and the need for parents and educators to invest heavily in educating achondroplastic children.

Roizen N, Ekwo E, Gosselink C. *Am J Med Genet* 1990;35:257–260.

Editor's Comment—*As pointed out by the authors, this is a small study, and the data are not sufficient to*

address certain issues, such as how well patients advance in their careers relative to their unaffected sibs. Moreover, the relatively low response rate, 23%, may have introduced bias into the results, eg, patients with low occupation scores may not have returned the questionnaires. Nevertheless, the final conclusion that education is a very important, if not major, determinant of adult success in the workplace is worth underscoring. This should be reassuring to average-statured parents of achondroplastic infants who are typically the most concerned about their child's occupational potential and what can be done to enhance it.

William A. Horton, MD

Table —Incidence of autoimmune diseases in PGAD/-I

	No. of Patients	Incidence %
Moniliasis (candidiasis)	68	100
Hypoparathyroidism	54	79
Hypoadrenalism	49	72
Diagnostic triad	35	51
Ovarian failure (age > 13 yr)	16 (of 29)	56
Testicular failure (adults)	3	12
Insulin-dependent diabetes mellitus	8	12
Pernicious anemia	9	13
Alopecia	20	29
Vitiligo	9	13
Keratopathy	24	35
Thyroid autoimmunity	3	4
Enamel hypoplasia	23	35
Calcified tympanic membranes	14/42	33
Ungual dystrophy (nails)	26/50	52
Mucocutaneous candidiasis	33/50	66

Clinical Variation of Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy in 68 Patients

This relatively rare entity, also called polyglandular endocrinopathy type I (PGE-I), is characterized by at least 2 of the following: hypoparathyroidism, hypoadrenalism, and mucocutaneous candidiasis. Other autoimmune diseases are often associated with this basic triad, including alopecia, pernicious anemia, gonadal failure, vitiligo, hypothyroidism or hyperthyroidism or chronic lymphocytic thyroiditis, and hepatitis. Ahonen et al have analyzed the interrelationships of these entities, as well as ungual dystrophy (pitted nails), steatorrhea, and keratopathy (Table).

Periodic malabsorption was observed in 12 patients, which was intensified with hypocalcemia, but some patients had steatorrhea when they were not hypocalcemic, and malabsorption preceded hypoparathyroidism in some. Neither keratopathy, pitted nails, nor enamel hypoplasia correlated with hypoparathyroidism or hypocalcemia, suggesting strongly that these were independent entities.

With the exception of candidiasis, none of these entities was manifest before the age of 12 months. Although most of the organ diseases occurred in childhood, some patients developed autoimmunity of some organs, including hypoparathyroidism, as adults. Interestingly, patients who developed Addison's disease as the first disease other than candidiasis tended to develop far fewer associated diseases.

Ahonen C, Myllarniessi S, Sipila I, et al. *N Engl J Med* 1990; 332:1829.

Editor's Comment—*This report greatly augments previous data on patients with PGE-I. The association of diseases and their time of appearance is surprising to me in that keratopathy and hypoplastic enamel are apparently not related to hypocalcemia or hypoparathyroidism. The relatively good prognosis of patients with only candidiasis and Addison's disease is worth noting. Unfortunately, the presumed autoimmune process in the gut that causes steatorrhea remains an enigma. Hypocalcemia, when present, needs to be controlled to minimize the steatorrhea. Interestingly, in the patients with Addison's disease, the aldosterone deficiency was evident before the cortisol deficiency in approximately 50% of the cases.*

The authors have published several previous reports of this syndrome, concerning mode of inheritance, oral findings, diagnosis and staging of hypocortisolism in progressive autoimmune adrenalitis, effective use of ketoconazole against candidiasis, the presence of adrenal and steroidal cell antibodies in evaluating risk of adrenocortical and ovarian failure, and the expression of PGE-I in association with human leukocyte antigen (HLA)-A, but not HLA-DR. The interested reader will find these and other pertinent references in the extensive bibliography of this article.

Robert M. Blizzard, MD

Prenatal Treatment of Females With Congenital Adrenal Hyperplasia Due to 21-Hydroxylase

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency has been well defined on a pathogenetic basis during the last few years. There are actually 2 genes linked to the HLA loci next to the C4B gene of the major histocompatibility complex on chromosome 6. Prenatal diagnosis is possible in the first trimester by chorionic villus sampling and DNA analysis or HLA linkage. Because congenital adrenal hyperplasia is the most common cause of female pseudohermaphroditism, the possibility of in utero therapy has been raised. This paper reports a pregnancy in which a female was recognized to be affected with the salt wasting form of congenital adrenal hyperplasia at 10 weeks of gestation. However, in order to suppress the adrenal, dexamethasone therapy had already been introduced during the eighth week. The child was born at term with minimal masculinization of her external genitalia in spite of being a severe salt loser.

The article summarizes the published total of 14 such cases of female infants who had been prenatally treated. Five newborn girls whose mothers received dexamethasone (starting between 5 and 8 weeks) had normal external genitalia. Five newborn girls whose mothers received hydrocortisone starting from 3 to 9 weeks had mild or partial virilization. Four female newborns whose mothers were treated with dexamethasone starting at 5 to 10 weeks had marked virilization.

It would appear that prenatal treatment has varying effectiveness. The reasons for this variation are not clear, but they may include familial variation in response to therapy, problems with transplacental passage of glucocorticoids, variations in maternal metabolism of glucocorticoids, variations in the clearance of exogenous glucocorticoids, fetal adrenal steroidogenic functional differences, and differences in the pituitary adrenal feedback mechanism.

Pang S, Pollack MS, Marshall RN, et al. *N Engl J Med* 1990;322: 111–115.

Editor's Comment—*This report demonstrates not only the power of DNA techniques to diagnose prenatally, but also the problems with intrauterine therapy: The therapy needs to be started earlier than it is possible to diagnose the presence of the biochemical abnormality. Since chorionic villus sampling is not available until approximately 9 weeks, the process of masculinization would have started prior to our ability to make the diagnosis. In addition, the range of masculinization among treated fetuses makes it clear that we do not really understand individual differences in response or the processes that lead to masculinization. It is clear that additional cases need to be followed carefully and reported so that we may ultimately arrive at the best therapies both in utero and ex utero. Follow-up information is also needed on those infants who are treated early but found not to be affected females. The assumption is that no harm has been done, but we need to be sure. A prospective collaborative study is very much needed. Hopefully, those pediatric endocrinologists with a special interest in congenital adrenal hyperplasia will establish such a study.*

Judith G. Hall, MD

Sex Steroids and Somatic Growth in Childhood

This is a short but provocative summary of the linear growth characteristics of 18 anatomically or functionally agonadal children with normal sex chromosomes, in an attempt to determine the importance of sex steroids to prepubertal growth. The children in this study included those with gonadal agenesis, gonadal dysgenesis, vanishing testes syndrome, surgical gonadectomy, gonadal destruction from radiation and chemotherapy, and biosynthetic defect in sex steroid production (17 α-hydroxylase deficiency). The agonadal status of these children was confirmed after surgical exploration, by determination of luteinizing hormone and follicle-stimulating hormone values, and plasma estradiol levels.

None of the 18 patients studied had heights or growth velocities greater than 2 SD below the mean of normal children. Thus, the authors suggest that gonadal steroids do not influence somatic growth during childhood and that it is highly unlikely that estrogen deficiency is responsible for growth failure of girls with Turner's syndrome.

Campos S, MacGillivray M. *Am J Dis Child* 1989;143;942-943.

Editor's Comment—*This short report is interesting and provocative. It stands, however, in sharp contrast to studies in pubertal children. To confirm these findings, it would be useful to evaluate a larger group of children, more homogeneous as to diagnosis. In addition, it is not clear that all the patients had completed their growth at the time they were studied. Hence, it would be useful to determine the effect of full physiologic replacement of sex steroids on final height in similar patients.*

William L. Clarke, MD

MEETING CALENDAR

October 16–20, 1990 The 41st Annual Meeting of the American Society of Human Genetics. Dr Albert B. Sabin Cincinnati Convention Center, Cincinnati, Ohio. Contact: American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

October 28–November 1, 1990 42nd Annual Postgraduate Assembly of the Endocrine Society. Sheraton Waikiki, Honolulu, Hawaii. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

January 9–12, 1991 38th Postgraduate Course, American Diabetes Association, Marriott Hotel and Marina, San Diego, Calif. Contact: American Diabetes Association, 1660 Duke St, Alexandria, VA 22314 (800-232-3472)

January 12–16, 1991 2nd International Symposium on Insulin-like Growth Factors/Somatomedins. The Grand Hyatt, Union Square, San Francisco, Calif. Contact: Sarah Burke, Extended Programs in Medical Education, Room C-124, University of California School of Medicine, San Francisco, CA 94143-0742. (Registration information 415-476-5808; program information 415-476-4251; fax: 415-476-0318)

February 6–9, 1991 Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, Calif. Contact: Marilyn Jones, MD, Children's Hospital, 8001 Frost St, San Diego, CA 92123 (619-576-5840)

February 9–13, 1991 18th Annual Seminar in Pediatric Nephrology: Current Concepts in Diagnosis and Management. Diplomat Resort and Country Club, Hollywood, Fla. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960, Miami, FL 33101 (305-549-6726)

March 16–21, 1991 Spring Session, American Academy of Pediatrics. San Diego Convention Center, San Diego, Calif. Contact: Department of Education, American Academy of Pediatrics, PO Box 927, Elk Grove Village, IL 60007 (800-433-9016)

April 29–May 30, 1991 Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Riverside Hilton, New Orleans, La. Contact: Society for Pediatric Research, 2650 Yale Blvd SE, Suite 104, Albuquerque, NM 87106 (505-764-9099)

May 12–15, 1991 International Symposium on Epidemiology and Etiology of IDDM in the Young. Chantilly-Gouvieux, France. Contact: Dr. Allen Drash, Children's Hospital, Pittsburgh, PA 15213

June 19–22, 1991 73rd Annual Meeting of the American Endocrine Society. The Sheraton Washington, DC. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (Tel: 301-571-1802; fax: 301-571-1869)

June 19–22, 1991 Combined ADA Council on Youth/ISGD Satellite Conference. "New Developments in the Etiology and Treatment of Childhood Diabetes." Williamsburg, VA. Contact: William L. Clarke, MD, Box 386, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908

June 24–27, 1991 30th Meeting of the Teratology Society. Boca Raton Club, Boca Raton, Fla. Contact: Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1841)

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Nontraditional Inheritance

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During the last few years, a body of evidence has accumulated suggesting that many kinds of genetic phenomena occurring are not explained by traditional Mendelian concepts. The purpose of this review is to bring to attention the genetic phenomena of mosaicism, uniparental disomy and genomic imprinting.

Mosaicism

A growing body of evidence suggests that *mosaicism* is a common, possibly universal phenomenon.[1] X-inactivation and tissue differentiation occur regularly, producing functional mosaicism. It has been calculated that all individuals must have some mutant cells.[1] Since the human body has approximately 10^{13} cells and since the mutation rate for known genes is in the order of 1 in 50,000, it follows that every individual will probably have mutations in some cells for every gene. Specific examples of mosaicism have been found in germ line and somatic cells now that DNA markers are available. For instance, there are individuals with neurofibromatosis who have a patchy or segmental form of neurofibromatosis and who appear to be somatic mosaics, but their affected children are fully affected in all cells, suggesting that the parent has both germ line and somatic mosaicism.[2] Similarly, there are known cases of several disorders where the parents appear clinically normal but have more than 1 affected child. This has been demonstrated on a DNA level in osteogenesis imperfecta[3]: 1 father was found to have 20% of his sperm carrying a gene for collagen with a specific deletion that led to perinatal lethal osteogenesis imperfecta. The accumulating evidence suggests that as many as 5% of new mutations are actually occurring while the parental germ line is developing. On a practical level, this means that the risk of recurrence in a "new" mutation may be much higher than previously predicted.[1] Similarly, it means that in rare situations in which there are 2 or more affected children with normal appearing parents, the affected children may represent a new dominant mutation in 1 parent's germ line rather than an autosomal recessive condition.

Chromosome mosaicism has been demonstrated for a long time but only recently has the recognition of patchy or streaky pigment as an indicator of mosaicism led to careful studies of fibroblast chromosomes in individuals with mental retardation and patchy pigment.[4,5] Thus, many individuals with hypomelanosis of Ito and a large number of patients with asymmetric growth have been found to be chromosomally mosaic.

Now that chorionic villus sampling is frequently being used as a means of prenatal diagnosis, it has been a suprise to find that as many as 5% of chorionic villus samples demonstrate chromosome mosaicism.[6] This suggests that chromosome mosaicism is a very common phenomenon during early embryonic development.

Uniparental Disomy

Uniparental disomy occurs when 2 copies of a particular chromosome come from 1 parent and none from the other parent. Isodisomy occurs where there are 2 copies of exactly the same chromosome. Heterodisomy is defined as the presence of 2 different copies in a chromosome pair. In uniparental heterodisomy they are both inherited from the same parent. With the new DNA markers it is possible to determine whether each of the 2 chromosomes of a particular pair are inherited from each parent as they usually are. To the surprise of most investigators many examples of uniparental disomy are being found. In 2 cases out of approximately 1,000 cases of cystic fibrosis, for example, both chromosome 7s have come from the mothers,[7,8] and

the fathers apparently are not carriers for cystic fibrosis. What this means is that the usual concept of an autosomal recessive disease, where both parents are carriers, may be incorrect for some cases of a particular disease. In other words, in these examples of cystic fibrosis, both copies of the abnormal gene came from the mother. This may be an explanation for "nonfamilial" recessive diseases. It seems likely that these cases of cystic fibrosis may have started as trisomy 7 until 1 of the chromosomes was lost, an event that actually allowed the survival of the individual since trisomy 7 is a lethal condition. Similarly, in the case of uniparental (maternal) disomy of chromosome 15, which produces Prader-Willi syndrome,[9] we have learned that an individual must have a paternal complement of 15q11-q13 to be normal. These cases probably started off as trisomy 15, which is lethal. If this assumption is correct, then survival occurs because 1 chromosome 15 is lost early in development. If after the loss 2 maternal chromosomes are left, the individual apparently develops Prader-Willi syndrome.

It is unclear how common a phenomenon uniparental disomy is. However, in specific disorders it may be a relatively frequent occurrence, eg, it accounts for 20% to 30% of Prader-Willi cases. There is reason to infer from research with mouse models that uniparental disomy of chromosome 7 in humans may be involved in producing the intrauterine growth retardation observed in the 2 cases of cystic fibrosis with uniparental disomy. Possibly, uniparental disomy is involved in other cases of intrauterine growth retardation or overgrowth.[10]

Genomic Imprinting

The concept of genetic imprinting holds that modifications of genetic material take place depending upon whether genetic information is derived from the mother or the father. These modifications are observed as differences in phenotype. An accumulating body of compelling evidence from research with animals and humans suggests that imprinting occurs in some parts of some chromosomes and to some genes, and thus must be taken into consideration when evaluating inheritance patterns in humans.[10]

The evidence for genomic imprinting includes observations made in mice reproduced by pronucleus transplantation or parthenogenesis. Zygotes are constructed in which all the genetic information comes from either the mother or the father.[11,12] These constructs are nonviable. Interestingly, when there is only paternally derived chromosome material, relatively normal development of membranes and placentas occurs, but very poor development of embryonic structures. In contrast, there is relatively good embryonic development but poor development of membranes and placentas in those zygotes with only maternally derived chromosomes. These 2 experimental situations are very similar to the naturally occurring human placental tumor, the hydatiform mole, in which there are 2 parental sets of chromosomes and overgrowth of the placenta, and also very similar to human ovarian teratomas, which are primitive tumors made up of all embryonic tissue types, but that are derived entirely from maternal chromosome complements.

The effects of genomic imprinting are also suggested by human triploids. Human triploids occur in 2 categories: those with 2 paternal chromosome sets and 1 maternal chromosome, and those with 2 maternal chromosome sets and 1 paternal. When there are 2 sets of chromosomes from the father, there is usually a typical well-grown cystic placenta (the typical cystic placenta of triploids) but poor embryonic growth. When there are 2 maternal complements, the pregnancy is almost always miscarried at an early stage, with very poor placental growth.[4] Therefore, paternal chromosomes appear to influence placental development more than maternal chromosomes and the reverse appears to occur in the development of the embryo.

Disomic experiments in mice support the concept of genomic imprinting.[13-15] Mice can be constructed where a segment of a chromosome or a whole chromosome will come only from 1 parent. A normal amount of chromosome material is present, but in a particular chromosome both copies will have been derived completely from either the mother or completely from the father. By working through the mouse chromosomes it has been found that there are 8 or 9 chromosome

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segments in which very different phenotypes are produced depending upon whether all the chromosome material comes from the male or female.[16] The changes that are seen are effects on growth, behavior, and survival.

As mentioned above, there are 2 human situations that are homologous. These are situations which have been recognized to represent uniparental disomy (ie, both chromosomes of a pair have come from 1 parent). There are 2 known cases of cystic fibrosis in which both chromosome 7s came from the mother. In these cases, the children with cystic fibrosis also have intrauterine growth retardation. There are also now several cases of Prader-Willi syndrome[9] that have occurred because the children have 2 chromosome 15s from the mother but no chromosome 15 from the father. Thus it appears that deficiency of a chromosome from 1 parent can lead to congenital abnormalities even though the correct number of chromosomes are present.

There are many chromosome deletion syndromes. Recently, it has been recognized that in the case of Prader-Willi and Angelman syndromes, it is usually the chromosome derived from a particular parent which is deleted. Prader-Willi and Angelman syndromes are deletions of the same area of chromosome 15. It is not clear whether they are deletions of exactly the same area of the chromosome; however, in the Prader-Willi syndrome, the chromosome 15 which is deleted is always the paternally derived one, and in the Angelman syndrome it is always the maternally derived one.[17] Interestingly, the "opposite" phenotypic effects seen in Prader-Willi and Angelman (hypotonic versus hyperactive) are very similar to those observed in the mouse disomy of distal chromosome 2.[16]

In a number of cases of congenital cancers, loss of heterozygosity is associated with the tumors but differential parental origin of the chromosome which is lost has been observed.[10] Thus, in sporadic Wilms' tumor, there is often loss of part or all of chromosome 11. It is almost always the maternal chromosome 11 that is lost.[18] These findings suggest that the maternal chromosome 11 plays some role in tumor suppression not compensated for by the paternal chromosome 11. Interestingly, familial Wilms' tumor is not linked to chromosome 11 but is usually transmitted by fathers. By contrast, examinations of sporadic sarcomas associated with loss of the retinoblastoma gene indicate that the chromosomal loss of chromosome 13 is almost always maternal.[20] However, in sporadic retinoblastomas of the eye, this kind of preferential loss from 1 parent or the other is not seen, suggesting that there may be differential imprinting in different tissues.

Transgene expression in some transgenic mice also seems to be modified depending upon the parent transmitting the gene in about a quarter of the cases examined.[11,21] In these situations, the DNA of the transgene has been integrated into the mouse genome and is passed from generation to generation, but the expression of the gene differs depending upon whether it is paternally or maternally transmitted. Interestingly, nonexpression is associated with methylation of the gene while expression is associated with nonmethylation, suggesting that the modifications are dependent upon methylation.[22]

Finally, there are a number of specific genes in humans and in mice in which there are unusual manifestations depending upon the sex of the parent from whom the gene is inherited.[10] For instance, in juvenile Huntington's disease, inheritance is almost always from the father; in the congenital onset form of myotonic dystrophy, inheritance is almost always from the mother. Seizures, cerebellar ataxia, spinocerebellar ataxia, Beckwith-Wiedemann syndrome, fragile-X syndrome, and neurofibromatosis type 2, all seem to have unusual preferential transmission expression.[10]

The concept of imprinting needs to be reexamined carefully and always considered when evaluating a particular disorder, since traditionally we have been trained to think that the sex of the parent of origin of a particular gene has no effect. It would appear from research with mice that as many as 10% to 20% of genes have the type of modification that depends upon the parent of origin. In human studies, it is important to reexamine specific diseases, chromosomal syndromes, and malformation syndromes in order to determine whether a differential parental effect is associated with severity, age of onset, and a particular manifestation. In particular, chromosome anomalies need to be reexamined to ascertain whether the differences in manifestations observed in a particular syndrome are actually related to parent of origin.

If one looks at the areas of human chromosome that are homologous to the areas of mice chromosome involved with genomic imprinting,[10] there are a number of very interesting human genes that lie in these areas, including genes having to do with atherosclerosis, gastrointestinal diseases, tumorigenesis, growth factors, and congenital anomaly syndromes. The possible role that genomic imprinting plays in humans with diseases related to these genes[10] is under study.

In summary, uniparental disomy occurs when both chromosomes of a pair or segments of

chromosomes of a pair are derived from the same parent. Several examples (Prader-Willi syndrome, 2 cases of cystic fibrosis, and sporadic cases of Wilms' tumor) have been cited. Imprinting is the modification of expression of genetic material that occurs depending upon whether the genetic material is derived from the mother or the father. For example, paternally derived material (genes) have a positive effect on placental development and maternally derived material (genes) have a positive effect on embryonal development. Combinations of mosaicism, uniparental disomy, and imprinting may explain a variety of conditions and unusual patterns of inheritance not previously understood in human disease processes.

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Mosaicism in Turner Syndrome

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As described by Henry Turner, the original clinical criteria for the diagnosis of Turner syndrome were: growth retardation, sexual infantilism, nuchal webbing and low posterior hairline, and cubitus valgus.[1] As others reported their experiences, the list of typical clinical features grew to include, among others: congenital lymphedema, a shield-shaped chest, multiple acquired and deeply pigmented nevi, cardiac and renal malformations, a high arched palate, recurrent otitis media, ptosis, short fourth metacarpals, and hypoplastic nails. The basis for the sexual infantilism was determined to be gonadal dysgenesis.[2] In 1959, Ford and colleagues demonstrated monosomy X in a typical patient.[3] Subsequently, many different karyotypic alterations were recognized to result in the clinical features of the Turner syndrome. Today, it is generally held that it is monosomy for the short arm of the X chromosome (in the absence of a normal Y) that is responsible for the phenotype we associate with the diagnosis of Turner syndrome. Deletions of the long arm will not be discussed in this review.

Among cytogenic surveys of liveborn individuals with the Turner syndrome,[4-7] a variety of chromosomal aberrations are reported with consistent frequency. Monosomy X (45,X) is found in approximately half of the patients with Turner syndrome. Patients with 46,X,i(Xq), in which there is a duplication of the long arm of one X and loss of the short arm, account for another 5%. In the remainder, there is mosaicism for 1 or more abnormal cell lines. An individual who is mosaic for a chromosomal abnormality has 2 or more cell lines that originate from a single zygote. This arises from mitotic nondisjunction occurring after fertilization. Patients who are mosaic for Turner syndrome may have a normal 46,XX cell line and a second population of 45,X, 46,X,i(Xq), 46,X,+r(X), or some other sex chromosome aberration. There may be mosaicism for 2 abnormal cell lines, eg, 45,X/46,X,i(Xq). Mosaicism for 3 or more cell lines also occurs. Some patients with Turner syndrome will be mosaic with a 45,X/46,XY or 45,X/46,X, or abnormal Y karyotype.

Clinical surveys of patients with Turner syndrome show that no single clinical feature is invariably present, although short stature is most constant. With a few exceptions, some of which are discussed below, there are no reliable pheno-

type-karyotype correlations in Turner syndrome, and the full-blown syndrome can be seen in patients mosaic with a normal cell line. Conversely, fertility and relatively normal stature in individuals with pure 45,X have been reported. These occurrences may possibly represent mosaicism only in the affected tissue and not in unaffected tissues.

While mosaicism for a normal or isochromosome X cell line is relatively common among live-born females with Turner syndrome, it is infrequently found in abortuses with X chromosome abnormalities.[8] It is the 45,X chromosome complement that is associated with high fetal mortality and that is responsible for 10% of recognized embryonic and fetal loss at 5 weeks of gestational age.[9] It is estimated that less that 1% of 45,X conceptuses survive to birth.[8] It has been suggested that all liveborn individuals with 45,X are mosaic to some degree for a cell line with 2 sex chromosomes, and that it is this occult mosaicism that has allowed them to survive. A single study[10] attempted to address this hypothesis. In none of 10 patients karyotyped (skin and blood) was a normal cell line detected. Nonetheless, it is still possible that the mosaicism exists in tissues other than peripheral lymphocytes or fibroblasts, that there is mosaicism limited to the placenta that allows for survival, or that a normal cell line is present long enough in embryogenesis to ensure that the infant is carried to term, and then is lost prior to birth.

The phenomenon of mosaicism in Turner syndrome presents several problems for the clinician. The first is the influence of a normal 46,XX cell line on phenotype and prognosis, ie, how hard should one search for a normal cell line? The second is the converse: how and when should mosaicism for an abnormal cell line be pursued in patients whose clinical findings suggest the clinical diagnosis of Turner syndrome and yet who appear karyotypically normal on initial testing of peripheral blood? Third, how should the presence of mosaicism for a 45,X cell line be interpreted in older women, given that loss of the second sex chromosome in tissue culture appears to be a normal feature of aging?[11] Fourth, mosaicism for part or all of the Y chromosome presents a unique problem in patients with Turner syndrome and is responsible for one of the few reliable phenotype-karyotype correlations in the disorder: the risk for gonadoblastoma. Finally, one other problem caused by mosaicism for sex chromosome aneuploidy is its significance when it is detected prenatally.

Mosaicism in Turner syndrome in which there is a 45,X cell line and a second cell line containing an X chromosome that is also abnormal, eg, 45,X/46,X,i(Xq);45,X/46,X+r(X), will not be addressed here. These patients do not differ phenotypically from 45,X individuals.

An adequate number of cells need to be evaluated to rule out mosaicism. Most laboratories will count between 25 and 50 cells to rule out mosaicism of 2% to 5% or more. Simpson[7] hypothesized that mosaicism for Turner syndrome is always detectable in blood and that karyotyping of fibroblasts is not necessary to detect a second abnormal cell line. Only 1 of our 131 patients who are mosaic for Turner syndrome is normal in blood and mosaic in fibroblasts. In all others, the mosaicism was detectable in peripheral lymphocytes. It is probably appropriate to discard the diagnosis of Turner syndrome if none of 50 lymphocytes counted is abnormal, and fibroblast karyotyping is probably unnecessary unless clinical findings for Turner syndrome are highly suggestive of the diagnosis.

Should one search for a normal cell line in patients with Turner syndrome and a 45,X karyotype?

The answer is "probably not." The presence of a 46,XX cell line in blood or in skin does not accurately predict taller stature, guarantee a better chance of fertility, or promise fewer complications of Turner syndrome. Although some reviews in the literature claim that 45,X/46,XX patients are generally more mildly affected,[4,13] we have not found this to be true in our patients (Tables 1 and 2). Although mosaics are more likely to have spontaneous menses, among our patients they are no more likely to be fertile. They are less likely to have congenital lymphedema and the physical features such as nuchal webbing and nail changes that are secondary to lymphedema. Lymphedema is far more common in infants with 45,X than in any other karyotype abnormalities for Turner syndrome. Thus, patients with 45,X are more likely to be diagnosed at birth than are other Turner syndrome patients, explaining the younger mean age of diagnosis in this group.

It is important to remember that the only persons who will come to medical attention for the diagnosis of Turner syndrome will be those who have some clinical stigmata to suggest the diagnosis. On the basis of current knowledge, it is useful, "if not necessary," to try to find a low level of mosaicism (less than 5%) for a normal 46,XX cell line in patients with Turner syndrome.

How important or useful is it to search for an abnormal chromosome complement in females in whom the question of the diagnosis of Turner syndrome is raised, but a cursory study of the karyotype is 46,XX?

It is reasonable to assume

Table 1

Characteristics	45,X (n=155)	45,X/46,XX (n=37)
Mean birth weight	2.928 kg	2.799 kg
Mean birth length	48.34 cm	47.82 cm
Prematurity	12%	10%
Mean age at diagnosis	8.3 yr	12 yr
Reason for diagnosis	40% Edema	3% Edema
	26% Short stature	33% Short stature
	19% Primary amenorrhea	25% Primary amenorrhea
	15% Other	39% Other
Final adult height	145.9 ± 4.7 cm	144 ± 5.6 cm

Table 2

Complications	45,X (n=155)	45,X/46,XX (n=37)
Mental retardation	12%	11%
Psychiatric disease	4%	8%
Edema	70%	28%
Web	51%	27%
Otologic	80%	80%
Ophthalmologic	54%	47%
Thyroid	17%	26%
Cardiac	58%	56%
Renal	52%	31%
Gastrointestinal	33%	47%
Spontaneous menses	9%	21%
Orthopedic	28%	47%

that there may be many women who have minor mosaicism for a 45,X cell line but are phenotypically entirely normal or might have a somewhat early menopause and/or subtle short stature who will not be identified as mosaics because the idea of karyotyping will never be entertained. One of our patients illustrates this point.

J.S. was initially referred at age 13 for short stature; she has been otherwise entirely healthy and had grown along the 50th percentile until age 9, when she fell below the third percentile. She had no other clinical features of the Turner syndrome. Her mother was 5 ft tall and her father was 6 ft tall. Mother's menarche was at age 14. Karyotyping revealed 45,X/46,XX(6%:94%) in peripheral lymphocytes. Thyroid functions, somatomedin C, and estradiol findings were all normal. She was lost to follow-up until she returned at age 18.5 years. Menarche had occurred at age 16; her menses were regular and she was 157.7 cm tall. A skin biopsy for karyotyping was obtained and revealed a normal 46,XX chromosome count. However, one of the X chromosomes appeared different from its homologue and a repeat blood sample was obtained to allow for better morphology. To our surprise, having assumed the original 45,X cell line was the result of an in vitro artifact, her repeat lymphocyte study again demonstrated mosaicism for 45,X in 2 of 50 cells (4%). Both X chromosomes were structurally normal. She has recently delivered her first healthy child.

The discovery of mosaicism in this patient was fortuitous and possibly irrelevant. Had her initial evaluation resulted in the clinical diagnosis of constitutional growth delay and chromosome testing had been deferred, her minor mosaicism would have remained undetected. Had her parents been less concerned, her short stature would not have been evaluated and would have resolved over time.

Among fertile patients with Turner syndrome, there is an increased risk for aneuploidy (both of the X chromosome and of the autosomes) in offspring. Is such a patient at increased risk? Horsman et al[13] karyotyped 100 women with repeated spontaneous loss of pregnancy and found 15 with mosaicism for 45,X (range, 2% to 10%). They found a similar proportion of 45,X mosaicism in women without a history of pregnancy loss and in none of their patients was a 45,X cell line detected in fibroblast culture.

Does a certain level of mosa-

icism for 45,X have to exist before a significant pregnancy risk is posed? Patients with Turner syndrome who have spontaneous menses often have premature ovarian failure or menopause. Is our patient at risk for this? There are no data to address these questions and it is clinically unwarranted to exhaustively search for minor mosaicism for 45,X in a girl with short stature or in a woman with gonadal dysgenesis and no other features of the Turner syndrome. Screening follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels is more likely to accurately measure ovarian function than the presence of minor mosaicism for 45,X and should be performed when appropriate.

How can one interpret mosaicism in older individuals?

In males and females alike, there is an increasing incidence of monosomy X in tissue culture with aging.[11] No studies have addressed the tolerable upper limits of mosaicism for 45,X in younger individuals. In the absence of clinical features, the diagnosis of Turner syndrome should not be made on the basis of minor mosaicism for 45,X cell line in women over the age of 30. As Horsman et al[13] suggested, the implications of mosaicism for childbearing are unclear and the results of karyotyping do not allow for discriminate predictions or management of patients.

How does mosaicism for a Y or Y-derived chromosome impact the management and treatment of the patient with Turner syndrome?

Mosaicism for 45,X/46,XY or 45,X/46,X, abnormal Y chromosome constitution is a special situation in Turner syndrome. These patients may be phenotypically classic for Turner syndrome or may be ascertained because of ambiguous genitalia or minor genital anomalies such as hypospadias and undescended testes. The presence of a Y chromosome confers an increased risk for gonadoblastoma (15% to 30%) in the gonadal streaks.[14-17] This risk increases with age. All patients with Turner syndrome and a Y chromosome should undergo prophylactic gonadectomy.

It has been suggested that those individuals with a nonfluorescent Y and/or those without certain repetitive Y-specific DNA sequences may not be at risk for gonadoblastoma. In at least 1 of our patients this was not true. Thus, until further studies are done or better methods of determining the presence of Y loci that confers the risk for malignancy are available, prophylactic gonadectomy in any patient with even a segment of a Y chromosome remains the appropriate treatment.

There have been a few reports of gonadoblastoma in individuals with 45,X Turner syndrome in whom there was no evidence of mosaicism for the Y chromosome.[18] Interestingly, at least 2 of the reported patients had spontaneous menses and ovulated for a number of years prior to gonadal failure. Katayama et al[18] suggested that spontaneous breast development might be a marker for those patients who were at risk for gonadoblastoma, but who did not have a Y chromosome or segment, because such occurred in his patients with gonadoblastoma and in some of those reported in the literature. This may not be a reliable marker, as we have seen spontaneous breast development (Tanner 2 and 3) in at least 10% of our adult patients with 45,X/46,X,i(Xq) and other "non-risk-bearing" karotypes, none of whom have developed gonadoblastoma. In addition, no breast development occurred in 5 of 8 cases of gonadoblastoma in non-Y-bearing women with Turner syndrome.[19-22] We believe some mild spontaneous breast development is not unusual in Turner syndrome and should not prompt gonadectomy in a non-Y-bearing individual. Gonadoblastoma also has been reported in patients who are mosaic for rings, markers, and fragments. It is often difficult to determine the chromosomal origin of a marker chromosome in patients with Turner syndrome. The assumption has been made that it is only those markers derived from a Y chromosome that confer an increased risk for gonadal malignancy. Until such time when molecular techniques for detecting those Y-specific sequences that confer the risk for gonadoblastoma[23] can be employed routinely and inexpensively in patients with Turner syndrome, management of patients who are mosaic for rings and fragments should be individualized. Routine screening with ultrasound imaging, prophylactic gonadectomy, or judicious nonintervention may all be appropriate.

What is the significance of prenatally detected mosaicism to the patient and the clinician?

Perhaps the most difficult problem posed by mosaicism for Turner syndrome is its prenatal detection. Mosaicism and pseudomosaicism in amniotic fluid cell cultures are common and may be found in 3.5% of samples.[24] When mosaicism for 45,X/46,XX or 45,X/46,XY is found at prenatal diagnosis, it presents significant problems in interpretation. The mosaicism may result from an in vitro artifact, may represent mosaicism limited to the placenta, or may be indicative of true fetal mosaicism. In a survey[25] of 92 cases of 45,X/46,XY mosaicism diagnosed prenatally, 76 were available for clinical examination either after termination or at delivery. Of these, 75 were phenotypic males and 1 was a

phenotypic female. Seventy-two of the 75 males had normal external genitalia. The remaining 3 males had hypospadias ranging from mild to severe. The single phenotypically female infant had clitoromegaly.

Gonadal histology was available for 11 of the fetuses following termination of the pregnancies. Of these, 3 abortuses with normal male external genitalia were found to have gonadal abnormalities. Several other surveys[26,27] also suggest that upwards of 90% of fetuses diagnosed as 45,X/46,XY will have a normal male phenotype at birth. This is in contrast to those patients diagnosed after birth who generally come to medical attention because they are phenotypically abnormal. As Chang et al underscored in their report,[24] dysgenetic gonads can occur in the presence of normal male external genitalia in 45,X/46,XY infants: "Therefore the risk of gonadal pathology is not limited to individuals with hypospadias or ambiguous genitalia." Even those infants who appear phenotypically normal continue to be at risk for gonadoblastoma and should be followed appropriately for that complication.

It will require long-term follow-up of these prenatally diagnosed cases to assess the lifetime risks of infertility, testicular failure,gonadoblastoma, and short stature. It is also clear from published surveys[25-29] that the proportion of karyotypically normal to abnormal cells in amniotic cell tissue culture does not predict the phenotypic outcome in the infant.

The same spectrum of clinically normal to classic dysmorphology of Turner syndrome occurs in mosaicism for 45,X/46,XX detected prenatally. At this time, mosaicism for sex chromosome aneuploidy that is detected prenatally continues to present a difficult counseling situation. Prospective parents need to be informed of the full range of possibilities. They need an explanation of the possible biases of prospective and retrospective studies and need to understand that there is no single "correct" response (ie, to terminate or to continue). It has been the experience in our clinic that most families consider many factors other than the specific phenotypic risk in arriving at a decision about pregnancy management. In nearly all situations, it has been possible to support the family in the decision they have already made. It is the exception rather than the rule that the information we give to families significantly changes their course of action.

Conclusions

In summary, the type of chromosomal abnormalities found in patients with Turner syndrome correlates poorly with the clinical findings, the possible exception being that the occurrence of gonadoblastoma in the primitive gonads is more likely to occur in patients with a Y chromosome. However, even this correlation is not absolute since patients with Turner syndrome and no Y chromosome or apparent fragment of a Y chromosome may develop gonadoblastoma. The statistics, however, do not justify routine gonadectomy in patients without a Y chromosome or fragment.

Mosaicism determined by amniocentesis may be misleading, as children without the usual characteristics of Turner syndrome have been reported to have a mosaic karyotype by amniocentesis. Counseling must be judicious when mosaicism of the X chromosome is found in specimens obtained by amniocentesis.

In contrast to beliefs of a year ago, the presence of XO/XY mosaicism does not consistently lead to abnormalities of the external genitalia.

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Several abstracts and presentations may be of interest to our readers. These involve the metabolic actions of GH and IGF-I, the relationships between GH, IGF-I, and diabetic complications, and the effect of diabetes on growth.

Fryburg and Barrett (*Diabetes* 1990;39[suppl 1]:115A) describe studies on the effect of GH on protein synthesis in the skeletal muscle of the isolated forearm of normal men. By using an infusion of GH (.014 μg/kg/min) and concomitant infusions of ^{3}H phenylalanine and ^{14}C leucine into the brachial artery for 6 hours, the synthesis and degradation of protein were determined at 3 and 6 hours. Systemic insulin, amino acids, glucose, and glucose uptake were unchanged during the study. A net anabolic effect on net forearm balance for both phenylalanine and leucine was observed due to an increase in skeletal muscle protein synthesis. Thus, GH apparently stimulates direct protein synthesis by skeletal muscle in a time-dependent manner in the absence of any change in plasma amino acids, and its action is distinct from that of insulin, which acts on primarily protein degradation.

Elahi et al (*Diabetes* 1990; 39[suppl 1]:88A) reported their studies on the acute effects of IGF-I infusions on glucose kinetics in healthy men. Utilizing the clamp technique, IGF was infused over 4 hours at 2 doses (75 and 112 μg/kg/4h) and compared to an insulin clamp (15 mU/m²/min). Glucose production declined equivalently in the 112 μg dose of IGF-I and the insulin clamp studies, but was unchanged when the 75 μg dose of IGF-I was infused by clamp. Glucose disappearance was increased by 165% by the 112 μg/kg dose and 70% by the 75 μg/kg dose. The larger dose suppressed insulin secretion without affecting GH levels. The authors state that these data indicate that there are different effects of IGF-I and insulin on the production and disappearance of glucose, with a more prominent effect of IGF-I on glucose disappearance. Thus, they speculate that IGF-I may be useful in a variety of insulin resistant states.

Agardh et al (*Diabetes* 1990;39[suppl 1]:31A) measured basal GH levels and TRH (200 mg) stimulated GH levels in 11 patients with type I diabetes who had rapidly progressive severe retinopathy. These were compared to those in a control group matched for age and duration of disease, but without background retinopathy or any retinopathy at all. Basal GH levels were above normal in the severe retinopathy group and higher than in the control group. In addition, the increase in GH levels after TRH was significantly higher in those individuals with severe retinopathy even though IGF-I levels were normal in all patients but one. The authors suggest that the results indicate that abnormal GH, but not IGF-I, may contribute to the development of severe retinopathy in type-I diabetic patients.

Werner et al (*Diabetes* 1990;39[suppl 1]:77A) measured the expression of IGF-I receptor genes in the kidney, brain, and testes of streptozocin-induced diabetic rats by determining the amount of ^{125}I-IGF-I binding to membrane preparations. The levels of mRNA for IGF-I receptor in the kidneys of diabetics were increased 2- to 3-fold as compared to controls, where no significant changes were detected in the levels of ligand mRNA for IGF-I binding. Insulin therapy reduced the levels of both receptor mRNA and binding to control values. There were no significant changes observed in the levels of receptor RNA or binding in either brain or testes. The authors suggest that the increased expression of IGF-I receptor in the kidneys of diabetics may explain, at least in part, the proliferation of mesangium in diabetic nephropathy.

A somewhat similar study was reported by Catanese (*Diabetes* 1990;39[suppl 1]: 11A), who studied IGF-I mRNA in streptozocin diabetic rats' livers, kidneys, and lungs. Untreated diabetic rats had a 10-fold reduction in hepatic IGF-I mRNA by 24 hours, whereas, a 2- to 3-fold increase of kidney IGF-I RNA was noted at 24 hours. This increase in kidney mRNA was not seen with doses of streptozocin less than 120 mg/kg, suggesting that the severity of the metabolic abnormality may affect this response. Lung IGF-I RNA levels were unchanged. Insulin therapy restored IGF-I mRNA levels toward normal.

The authors state that these data suggest that gene expression for IGF-I is regulated in a tissue-specific manner in diabetes and that factors in addition to GH may modulate the endocrine effects on growth.

Wise et al (*Diabetes* 1990; 39[suppl 1]:29A) reported on a longitudinal study of the growth velocity in 112 children with diabetes. The children were seen at 4-month intervals for a total of 715 visits. Glycemic control was measured by determining glycosolated hemoglobin concentrations (normal, 4% to 8%). Pubertal status was determined by

physical exam, and the height was measured utilizing a stadiometer. Height measurements were normalized for age and sex by converting to Z scores. A linear relationship was seen between glycosolated hemoglobin concentrations and delta Z (r= -0.15, P <0.001). Glycosolated hemoglobin values less than 8% were associated with growth acceleration whereas the greatest growth deceleration occurred with HbA_1 greater than 16%. The level of glycosolated hemoglobin at which growth suppression occurred was dependent on pubertal status: Tanner 1, $HbA_1 \geq 10\%$; Tanner 2 to 3, $HbA_1 \geq 8\%$; Tanner 5, $HbA_1 \geq 16\%$. The authors concluded that linear growth velocity is closely related to metabolic control, and that children in early puberty appear to be the most vulnerable to growth suppression when control is poor. Once puberty is well established, growth suppression does not occur until marked hyperglycemia is evident.

William L. Clarke, MD

In Future Issues

Obesity in Childhood and Adolescence, Part I: Genetics, Physiology, and Growth
by W.H. Dietz, MD, and L.G. Bandini, MD

Obesity in Childhood and Adolescence, Part II: Pathophysiology, Associations, and Complications
by W.H. Dietz, MD

Oxandrolone Therapy: 25 Years Experience
by R.M. Blizzard, MD, P.C. Hindmarsh, MD, and R. Stanhope, MD

Update: The Genetics of Insulin-Dependent Diabetes
by W.E. Winter, MD, and N.K. McLaren, MD

Support Groups for Individuals With Growth Problems and Their Families
by J. Weiss, MSW, LCSW, and J.G. Hall, MD

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Abstracts From the Literature

Effect of Puberty on Initial Kidney Growth and Rise in Kidney IGF-I in Diabetic Rats

Bach and Jerums studied the development of kidney enlargement and insulin-like growth factor I (IGF-I) levels in kidney tissue in prepubertal and postpubertal male Sprague-Dawley rats, half of whom were made diabetic by streptozocin (STZ) injections. On days 1 through 3 and day 7 after STZ injection, groups of diabetic and control animals were sacrificed following blood sampling for plasma IFG-I, testosterone, and glucose determinations. Plasma IGF-I and testosterone levels were significantly higher in postpubertal rats. The pattern of kidney enlargement was different despite comparable blood glucose levels in prepubertal and postpubertal diabetic rats. Kidney weight increased significantly more in postpubertal diabetic rats than in postpubertal controls by day 2 after STZ injection. By day 7, the kidney weight had increased by 36%. In contrast, kidney weights of diabetic

prepubertal rats became significantly greater (by 14%) than that of prepubertal controls on day 7 only. The content of IGF-I in kidneys was significantly greater in postpubertal diabetic rats than in controls, with a peak at days 1 and 2 following STZ injection. In contrast, kidney IGF-I levels in prepubertal diabetic rats were no different from the controls. Because of these findings, the authors suggest that local accumulation of IGF-I may be important in kidney enlargement associated with diabetes.

The authors state that kidney enlargement is a well-described early feature of insulin-dependent diabetes in humans as well in STZ-injected rats. This increase in kidney size in humans is associated with an increase in glomerular filtration and may be associated with an increased risk of developing diabetic nephropathy. Thus, the rise in kidney IGF-I that precedes kidney growth in postpubertal diabetic rats in this study is consistent with a potential role for local IGF-I accumulation in diabetes-associated kidney enlargement. In addition, the differences observed between the kidney weights of prepubertal and postpubertal diabetic rats are compatible with the hypothesis that nephropathy in human diabetes is a postpubertal event.

Bach LA, Jerums G. Diabetes 1990;39:557-562.

Editor's Comment: *Although it is not the policy of Growth, Genetics, and Hormones to abstract manuscripts dealing with animal studies, the present study has such important implications for understanding the relationship between diabetic nephropathy and puberty that it has been included here. It has been suggested that diabetic complications are related to the postpubertal duration of the disease. Several investigators have reported that microalbuminuria, an early sign of nephropathy, never occurs prepubertally; and capillary basement membrane thickening, which is another feature of diabetic microangiopathy, has been shown to be related to the level of glucose control, but only in postpubertal subjects. The data in the present study affirm these clinical findings in that kidney enlargement was observed in postpubertal animals. In addition, the observation that kidney IGF-I levels correlate with kidney weights in these diabetic animals probably is an important observation. Other investigators have demonstrated that the administration of somatostatin analogue can suppress kidney growth in diabetic rats, which suggests a role for IGF-I in the process of kidney growth and in the development of diabetic nephropathy.*

William L. Clarke, MD

Effect of the Long-Acting Somatostatin Analogue SMS 201-995 on Growth Rate and Reduction of Predicted Adult Height in Ten Tall Adolescents

Tauber et al studied the use of SMS 201-995 as therapy for tall stature in 10 patients (4 boys and 6 girls), aged 11.5 to 17 years, whose mean height deviation was +3.2 standard deviations. Patients were eligible for the study if they were over 11 years old (girls) and 13 years old (boys) and had a predicted adult height of at least 190 cm (boys) and 180 cm (girls) according to Bayley and Pinneau tables. Mean bone age (BA) for boys was 14.6 years and for girls 12.4 years. SMS 201-995 (250 U) was given twice daily (at 0700-0800 hours and 30 minutes prior to bedtime). Six patients were treated for 1 year and 4 patients were treated for 6 months. Therapy was stopped in boys at a BA of 17 years or greater and in girls at a BA of 15 years. Height and weight measurements were performed every 45 days. Somatomedin C levels were measured at 0, 3, 6, and 12 months of therapy. BA was evaluated at 6 and 12 months. Patients underwent 24-hour growth hormone (GH) evaluation, and integrated concentrations of GH were calculated. Mean growth rates significantly decreased from 7.1 cm/yr to 2.7 and 2.4 cm/yr after 6 and 12 months of therapy, respectively. Mean BA increased from 14.6 years before therapy to 15.8 and 16.8 years, respectively, after 6 and 12 months of therapy. Delta SDS/BA before and after 6 and 12 months of SMS 201-995 therapy are shown in Table 1. The 24-hour mean integrated concentration of GH decreased from 5.3 to 3.6 ng/mL per minute after 6 months and to 3.9 ng/mL per minute after 12 months of therapy, although individual responses were highly variable.

Somatomedin C values decreased from 1.7 to 0.9 U/mL after 3 months and to 1.1 and 1.0 U/mL after 6 and 12 months, respectively. Mean predicted adult height decreased from 198.7 to 193.7 cm in boys and from 184.5 to 179.7 cm in girls. Final height has not been

Table 1 — Growth rate SDS/BA before and after 6 and 12 months of SMS 201-995 therapy

	Before SMS 201-995 SDS/BA	6 months SDS/BA	12 months SDS/BA
Patients			
1	-0.6	-2.2	-0.4
2	+0.2	-1.5	—
3	+0.3	0.6	-1.7
4	+0.4	-2.5	-0.4
5	+0.9	-2.9	-1.4
6	+0.5	-3.4	-2.2
7	+0.7	-1.7	0
8	+0.2	-3.3	—
9	+0.1	0	—
10	-0.6	-2.2	—
Mean	+0.4	-2.0	-1.0
SD	0.2	1.1	0.9

achieved in all cases. No patient discontinued SMS 201-995 because of side effects, although transient diarrhea was noted in all cases during the first 10 days of treatment. Routine blood chemistries; complete blood count; vitamin A, D, and E levels; and glycosolated hemoglobin remained normal in all patients. Other endocrine functions remained within the normal range. Menarche occurred during SMS treatment in 4 girls. One patient developed asymptomatic gallbladder microlithiasis at 6 months of treatment.

Tauber MT, Tauber JP, Vigoni F, et al. *Acta Paediatr Scand* 1990; 79:176-181.

Editor's Comment: *SMS 201-995 administration was associated with decreases in growth rate and plasma somatomedin C levels in this study. The authors stated, however, that the response to treatment was variable and unpredictable. This is an important study, as the routine use of ethinyl estradiol in tall girls may be associated with significant hyperlipidemia. SMS was apparently well tolerated during this study, but as the authors point out, the minimum effective daily dose has not been defined. In addition, there were no controls in this study. It would have been useful to have evaluated adolescents or children with similar BAs and similar predicted heights who received no therapy or received traditional estrogen or testosterone therapy. Thus, one cannot conclude that the reduction in growth velocity is different from that which may have occurred with other therapies. In addition, the patients in this study had relatively advanced BAs, and all were either in Tanner stage 3 or 4. It is conceivable, then, that their growth rates may have declined during the year of therapy regardless of the use of SMS. Despite the drawbacks of this study, the information presented suggests that long-acting somatostatin analogues may be useful for the treatment of tall stature.*

William L. Clarke, MD

Effect of Oral Clonidine Insulin-Induced Hypoglycemia and Exercise on Plasma GHRH Levels in Short Children

The ability to measure radioimmunoassayable growth hormone releasing hormone (GHRH) in the peripheral blood offers some insight into the mechanisms of action of the various stimuli that are used for routine evaluation of the secretion of growth hormone (GH) in children. A previous work (Donnadieu M, Evain-Brion D, Tonon MC, et al. *J Clin Endocrinol Metab* 1985;60:1132-1134) had shown that L-dopa increases blood GHRH just before the GH peak while ornithine or arginine infusion does not. The current study (Gil-Ad I, Leibowitch N, Josefsberg Z, et al. *Acta Endocrinol* 1990;122:89-95) extended this type of investigation to 3 other GH stimulation tests.

Thirty-one healthy short stature children in whom GH deficiency had been ruled out underwent 1 of the following tests: oral clonidine, 0.15 mg/m^2 (n = 13); insulin-hypoglycemia, 0.1 U/kg IV (n = 12); or exercise (n = 6). Their GH peaks during these tests were in the normal range. Clonidine induced a significant increase of peripheral GHRH levels from 5.6 ± 1.5 pmol/L at the basal level to 12.2 ± 2.5 pmol/L at 60 minutes. Neither insulin-induced hypoglycemia nor exercise signif-

icantly changed the plasma levels of GHRH. This clearly suggests that clonidine provokes a release of GH through GHRH, whereas stress stimuli such as hypoglycemia and exercise achieve GH release in other ways – possibly inhibition of somatostatin.

Moreover, the current study was extended to determine the effects of clonidine and hypoglycemia upon the thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH). Clonidine did not modify it. Insulin provoked a potentiation of the total response and an anticipation of the TSH peak following TRH injections. Since it is known that somatostatin inhibits the TSH-releasing effect of TRH, these results favor the hypothesis of an inhibition of somatostatin during insulin-induced hypoglycemia.

Editor's Comment: *The various ways of evaluating the secretion of GH and/or the releasable pituitary GH stores continue to deserve attention. It could be of physiologic and practical importance to know the sites at which the different stimuli in current clinical use act upon the complex regulatory mechanisms that command the release of GH. Measuring GHRH in peripheral venous blood, although it cannot reflect exactly what happens in the hypothalamo-hypophyseal portal circulation, offers an approach to its study in humans. The parallels between the studies cited here prompt me to point out the similarity between the action of clonidine and that of L-dopa, which both increase not only plasma GH but also GHRH, whereas infusion of amino acids or stress tests do not. This also emphasizes the importance of somatostatin in the regulation of GH secretion, which up to now has not been possible to investigate directly in children but could play a major role in various growth disorders and especially in functional or so-called idiopathic GH deficiencies.*

Jean-Claude Job, MD

Editor's Comment: *Measurements of GHRH in the peripheral circulation are somewhat difficult to interpret, as GHRH may be produced outside the hypothalamus. The importance of these observations depends upon unequivocal certainty that the GHRH measured in the peripheral circulation reflects what takes place in the hypothalamus. Confirmation of this may be difficult. The topic will be followed with much interest by all of us.*

Robert M. Blizzard, MD

The Prepubertal Hiatus in Gonadotropin Secretion in the Male Rhesus Monkey *(Macaca mulatta)* Does Not Appear to Involve Endogenous Opioid Peptide Restraint of Hypothalamic Gonadotropin-Releasing Hormone Release

In higher primates, gonadotropin secretion is elevated in early infancy and again in puberty, but between these times there is a period extending from 6 to 30 months in Rhesus monkeys in which pulsatile gonadotropin-releasing hormone (GnRH) release essentially ceases. Since it has been demonstrated that GnRH neurons of prepubertal monkeys receive innervation from endogenous opioid peptide (EOP) neurons, experiments were done to determine if the restraint of the GnRH secretion was due to EOP secretion.

The EOP antagonist, naloxone, was given to a number of castrated monkeys in 3 doses: as a bolus, as a continuous infusion, and as an intermittent infusion. Prior to the naloxone, the monkeys had 3 weeks of intermittent GnRH infusion so that the pituitary was appropriately primed to respond. However, the naloxone did not cause any increase in blood luteinizing hormone (LH) although a GnRH injection immediately after the experiment always produced high responses. Thus, the mechanism of the childhood GnRH restraint does not involve EOPs. The authors add that unpublished experiments in female monkeys show the same effect.

Medhamurthy R, Gay VL, Plant TM. *Endocrinology* 1990;126:1036-1042.

Editor's Comment: *This characteristically well-designed experiment has a very clear cut result that has been already adumbrated in the human by Mauras, Veldhuis and Rogol (J Clin Endocrinol Metab 1986;62:1256-1263). The mechanism of the GnRH restraint—enormously important from an evolutionary point of view—remains entirely unknown.*

James M. Tanner, MD

The Predictive Value of Short-Term Growth Using Knemometry

Seventy-eight normal school children aged 3 to 16 years were measured with the knemometer at 1, 2, 3, 6, 9 and 12 months. Height was also measured, in the evening between 1800 and 2100 hours. The error of knemometry was 0.18 mm and of height measurement was 0.70 mm. Month-to-month variability in leg-length velocity averaged 2 mm with a range of 1 to 4 mm among these individuals whose monthly mean growth rate was 1.6 mm. The correlation between growth over 1 month and over 12 months in leg length was virtually zero. Over 6 months to 12 months, it was .84. The correlation between height measured over 12 months and leg length over 1 month was .3; leg length over 3 months, .66, over 6 months, .85 and over 12 months, .89. The authors conclude that a knemometric rate calculated over less than 6 months is useless for assessing what the annual growth rate will be.

Dean HJ, Schentag CT, Winter JSD. *Acta Paediatr Scand* 1990;79:57-63.

Editor's Comment: *This is a very welcome independent confirmation of the values recently published by Hermanussen and his associates, and confirmed by Wales and Milner in 1987. It is true that the height gain over 12 months was a little better predicted by the leg-length gain over 6 months than by the height gain over 6 months, but this is probably because the error of height measurements is unacceptably high, whereas the error of knemometry is absolutely in line with Hermanussen's values. (It has long been noted by anthropometrists that familiarity breeds contempt.)*

James M. Tanner, MD

Long-Term Treatment With Glucocorticoids/ACTH in Asthmatic Children

Forty children born between 1947 to 1974 with bronchial asthma severe enough to require long-term treatment with glucocorticoids or ACTH have been followed, 31 until adult height was reached. Twenty-three were given prednisolone for an average of 6.5 years beginning at an average age of 6 years, and 17 were given daily ACTH for 3 years, starting at an average of 5.5 years. The prednisolone-treated group had a height SDS of -1.0 at the beginning of treatment, -1.4 after 1 year, -1.8 after 2 years, and -2.4 after 3 years. In contrast the ACTH group, starting at -0.5, after 1 year were -0.1, after 2 years were +0.2, and after 3 years were +0.2. Thus, the height velocity for the ACTH-treated group was at all times above the mean, whereas for the prednisolone-treated group, it was at all times well below the mean. The diminished velocity on prednisolone was not significantly dose-related and was present in doses as small as 0.1 mg/kg per day.

The adult height of the ACTH-treated group was well within normal limits, as was their age at peak height velocity, whereas the adult height of the prednisolone-treated group was more than 2 SD below the mean in boys and approximately 1.5 SD below the mean in girls. Age at peak height velocity was severely retarded in the boys, by approximately 2 SD, whereas it was not so in the girls, whose age at menarche was within normal limits.

Oberger E, Engstrom I, Karlberg J. *Acta Paediatr Scand* 1990:79;77-83.

Editor's Comment: *This paper makes a very strong argument for treatment with ACTH rather than with prednisolone. The authors' conjecture is that the retardation in puberty in boys is due to long-term glucocorticoid effect on testosterone levels. Since other series report a normal adult height in patients despite glucocorticoid treat-ment, it is perhaps important to terminate glucocorticoids well before the expected time of puberty to allow some degree of catch-up.*

James M. Tanner, MD

Growth of African Pygmies in Early Childhood

Growth curves are given for the height of Efe pygmy children from 6 months to 5 years of age. The data are mixed longitudinal; all dates of birth were known. At 6 months of age, the mean height standard deviation score, relative to National Center for Health Statistics (NCHS) standards, was -2.7, declining to -4.2 at 5 years of age. The mean score for adults, sexes pooled, was -4.8 SD. Thus, most of the pygmy height deficit is accrued by 5 years of age.

Bailey RC. Letter to the editor. *N Engl J Med* 1990;323:1146.

Editor's Comment: *This letter to the editor is of importance because Merimee, et al* (N Engl J Med *1987;316:906-911) suggested that the short stature of adult pygmies is due primarily to a deficient growth spurt during puberty. In the article by Merimee, et al curves for cross-sectional height increment were given for pygmies that appear to show that male pygmies have no pubertal growth spurt, while female pygmies appeared to have a very reduced pubertal growth spurt. Merimee, et al reported that the testosterone values were normal at all ages, while IGF-I levels failed to rise to the same extent in pygmies at adolescence (250 U/mL) vs 500 U/mL in American adolescents.*

As stated in the abstract of the article by Bailey, most of the pygmy height deficit is determined to accrue by 5 years of age when he studied pygmy infants and children. The question now is why did Merimee, et al conclude that there is little adolescent growth spurt in pygmies. No other human group has such a lack of pubertal growth spurt (see Eveleth and Tanner[1]). The answer to the question probably is that very few adolescents were measured by Merimee, et al and their conclusion was actually derived from the report of J.M.H. van de Koppel and B.S. Hewlett in a 1986 book called African Pygmies.[2] *Between 1975 and 1980, the authors of this report measured the heights of 307 Akan pygmies whose ages had been estimated by means of an event calendar — a standard, though imperfect technique, listing major events concerning the tribe back into the past, utilizing the mother's input regarding when a child was born (eg, before or after each of those events). The report contains no tables of value: however, by using the graphs it is possible to estimate that about 50 persons of each sex were probably measured during the pubertal age range. It is quite likely, therefore, that had puberty stages been determined for each, the pattern of mean height increments between those in stages 2 and 3, 3 and 4, and 4 and 5 would have revealed, at least in the boys, whether or not a pubertal growth spurt occurred. Unfortunately, this was not done.*

Instead, an exponential curve was fitted to all the data from birth to adulthood. Though the authors suggest the curves explain "more than 99% of the variance," I believe they have confused "within-age variation" with "between-age variation." In fact, there is a great excess of males above the curve at ages 10 to 16 years. The authors also provide plots of approximately year-to-year mean increments calculated cross-sectionally, and these curves permit a reasonable judgment. Female pygmies appear to have a maximum mean increment of about 9.5 cm/yr, which is above the cross-sectional population mean increment for American girls at puberty. Male pygmies seem to have a maximum growth increment of about 6.5 cm/yr, which is slightly below the Western mean increment value of approximately 7.2 cm/yr. Both values are well within the usual sampling limits, given the small numbers.

The plots of annual mean increments in the New England Journal of Medicine *article give a very inaccurate impression. The authors have taken the British longitudinal, tempo-conditional mean velocities with their big peaks, and plotted cross-sectional population values (grossly smoothed) upon them, evidently unaware of the differences (see Tanner[3]). The authors' contention regarding the lack of a pubertal growth spurt remains unproven, and, sad to say, this article is yet another example of biochemical expertise combined with auxologic innocence.*

Bailey's article, in contrast, is a very clear and unexceptionable statement regarding the early growth of the Efe pygmies. He continues his longitudinal studies there, and the results through puberty will be awaited with interest.

James M. Tanner, MD

References

1. Eveleth FB, Tanner JM. *Worldwide Variation in Human Growth.* 2nd ed. Cambridge, England: Cambridge University Press; 1990.

2. van de Koppel JMH, Hewlett BS. Growth of Akan pygmies and Bagandus of the Central African Republic. In: Cavalli-Sforza LL, ed. *African Pygmies.* New York, NY: Academic Press; 1987: 95-102.

3. Tanner JM. The use and abuse of growth standards. In: Falkner F, Tanner JM, eds. 2nd ed. *Human Growth* New York, NY: Plenum; 1986;3:280-285.

MEETING CALENDAR

January 9-12, 1991 38th Postgraduate Course, American Diabetes Association, Marriott Hotel and Marina, San Diego, CA. Contact: American Diabetes Association, 1660 Duke St, Alexandria, VA 22314 (800-232-3472)

January 12-16, 1991 2nd International Symposium on Insulin-like Growth Factors/Somatomedins. The Grand Hyatt, Union Square, San Francisco, CA. Contact: Sarah Burke, Extended Programs in Medical Education, Room C-124, University of California School of Medicine, San Francisco, CA 94143-0742. (Registration information 415-476-5808; program information 415-476-4251;fax 415-476-0318)

January 27-February 1, 1991 Advances in Gene Technology: The Molecular Biology of Human Genetic Disease. Information: The Miami Bio/Technology Winter Symposia, PO Box 016129, Miami, FL 33101-6129. (Tel: 800-642-4363; fax: 305-324-5665)

February 6-9, 1991 Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, CA. Contact: Marilyn Jones,MD, Children's Hospital, 8001 St, San Diego, CA 92123 (619-576-5840)

February 9-13, 1991 18th Annual Seminar in Pediatric Nephrology: Current Concepts in Diagnosis and Management. Diplomat Resort and Country Club, Hollywood, FL. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960 Miami, FL 33101 (305-549-6726)

March 16-21, 1991 Spring Session, American Academy of Pediatrics, San Diego Convention Center, San Diego, CA. Contact: Department of Education, American Academy of Pediatrics, PO Box 927, Elk Grove Village, IL 60007 (800-433-9016)

March 16-23,1991 3rd International ISGD Course on Update on Diabetes in Childhood. Maega Ciapela, Marmolada, Italy. Information: Dr. L. Pinelli, Servizio di Diabetologia Pediatrica, Policinico 1-37134 Verona, Italy. (Tel: 39-45-933-667; fax: 39-45-8200-993.)

March 17-20, 1991 5th European Workshop on Pituitary Adenomas: New Trends in Basic and Clinical Research, Venice, Italy. Program Information: Dr. C. Faglia, Inst. of Endocrine Sciences, Univ. of Milan, Via F. Sforza 35, I-20122, Milan, Italy. Tel: 39-546-4063. General Information: M. Volpl/A. Cogo, Deltagest, Via E. Toti 9, I-35135 Padova, Italy. (Tel: 39-49-600-288)

April 10-12, 1991 International Symposium on Growth Disorders: The State of the Art. Bari, Italy. Contact: Serono Symposia, Via Ravenna 8, 00161 Rome, Italy.

April 26-27, 1991 KABI 11th International Symposium on Growth and Growth Disorders, Stockholm, Sweden. Information: Dr. R. Gunnarsson, Kabi Vitrum Peptide Hormones, S-11287 Stockholm, Sweden. (Tel: 46-8-138-000; fax 46-8-618-2019)

April 29-May 30, 1991 Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Riverside Hilton, New Orleans, LA. Contact: Society for Pediatric Research, 2650 Yale Blvd SE, Suite 104, Albuquerque, NM 87106 (505-764-9099)

May 1-3, 1991 Annual Meeting of the LWPES. New Orleans, LA. Information: Dr. G. August, Secretary, LWPES, Children's National Medical Center, 111 Michigan Ave, NW, Washington, DC 20010. (Tel: 202-745-2121; fax: 202-939-4492)

May 24-26, 1991 International Symposium on Growth and Development: Basic and Clinical Perspectives, Auckland, New Zealand. Information: Prof. P. Gluckman, Dept of Pediatrics, Univ of Auckland, Private Bag, Auckland, New Zealand. (Tel: 64-9-795-780; fax: 64-9-770-956)

May 12-15, 1991 International Symposium on Epidemiology and Etiology of IDDM in the Young. Chantilly-Gouvieux, France. Contact: Dr. Allen Drash, Children's Hospital, Pittsburgh, PA 15213

June 19-22, 1990 17th Annual Meeting of the ISGD, Williamsburg, VA. Information: Dr. W. Clarke, Dept of Pediatrics, Box 386, Univ of Virginia Health Sciences Center, Charlottesville, VA 22908. Tel: 804-924-5897; fax: 804-924-2769. Registration via ADA, 1600 Duke Street, Alexandria, VA 22314. (Tel: 703-836-1500; fax: 703-836-7493)

June 19-22, 1991 73rd Annual Meeting of the American Endocrine Society. The Sheraton, Washington DC. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (Tel: 301-571-1802; fax: 301-571-1869)

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